RECOMBINANT DNA RESEARCH
Volume 19

Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules" January 1994–August 1994

December 1995

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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Volume 19

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Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules" January 1994–August 1994

December 1995

Prepared by the
Office of Recombinant
DNA Activities

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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PREFACE

This is the nineteenth in a series constituting a "public record" of activities in regard to the National Institutes of Health (NIH) Guidelines for recombinant DNA research.

The first eighteen volumes cover events from February 1975 through December 1993. This nineteenth volume covers events from January 1994 through August 1994.

Starting from Volume 14, human gene transfer/therapy protocols that have been approved by the NIH Recombinant DNA Advisory Committee, are collected. Documents of each human study include the clinical protocol, the Informed Consent document describing the study in a nontechnical language, both scientific and nontechnical abstracts, and curriculum vitae of the principal investigators.

Volumes 1 through 5 in this series and the Environmental Impact Statement may be purchased from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, DC 20402 or GPO bookstores in selected cities throughout the United States. They may also be viewed in some 600 public libraries of the GPO depository system. The GPO stock numbers are: Volume 1, 017-004-00398-6; Volume 2, 017-040-00422-2; Volume 2 Supplement (Environmental Impact Statement), 017-040-00413-3; Volume 3, 017-040-00429-0; Volume 3 Appendices, 017-040-0043-3; Volume 4, 017-040-00443-5; Volume 4 Appendices 017-040-00422-7; and Volume 5, 017-040-00470-2. Volumes 6 through 19 are available from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD 20892, USA.

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federal register

Friday
February 11, 1994

Part III

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under the Guidelines and Meeting; Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on March 3–4, 1994. The meeting will be held at the National Institutes of Health, Building 31C, 6th Floor, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on March 3, 1994, to adjournment at approximately 5 p.m. on March 4, 1994. The meeting will be open to the pubic to discuss Proposed Actions under the NIH Guidelines for Research involving Recombinant DNA Molecules (51 FR 16958) and other matters to be considered by the Committee. The proposed Actions to be discussed will follow this notice of meeting.

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20902, Phone (301) 496–9838, FAX (301) 496–9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: February 7, 1994.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 94–3179 Filed 2–10–94; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Research: Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on March 3–4, 1994. After consideration of these proposals and comments by the RAC, the Director of the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by February 24, 1994, will be reproduced and distributed to the RAC for consideration at its March 3–4, 1994, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301–496–9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules:

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Hersh, Akporiaye, Harris, Stopeck, Unger, and Warneke

On December 23, 1993, Dr. Evan Hersh of the Arizona Cancer Center and Drs. Akporiaye, Harris, Stopeck, Unger, and Warneke of the University of Arizona, Tucson, Arizona (co-sponsored by Vical, San Diego, California), submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Immunotherapy of Malignant Melanoma by Direct Gene Transfer.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Walker

On January 4, 1994, Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland (co-sponsored by Cell Genesys, Foster City, California), submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T-Lymphocytes in HIV-Infected Identical Twins.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Rosenblatt and Seeger

On January 5, 1994, Drs. Joseph Rosenblatt of the University of California, Los Angeles, California, and Robert Seeger of the Childrens Hospital, Los Angeles, California, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Study of Immunization with Gamma Interferon Transduced Neuroblastoma Cells.

IV. Addition to Appendix D of the NIH Guideline Regarding a Human Gene Transfer Protocol/Dr. Brigham

On January 6, 1994, Dr. Kenneth Brigham of Vanderbilt University, Nashville, Tennessee, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Expression of an

Exogenously Administered Human Alpah-1 Antitrypsin Gene in the Respiratory Tract of Humans.

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Freedman

On January 5, 1994, Dr. Ralph Freedman of the MD Anderson Cancer Center, Houston, Texas, resubmitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Use of a Retroviral Vector to Study the Trafficking Patterns of Purified Ovarian Tumor Infiltrating Lymphocytes (TIL) Used in Intraperitoneal Adoptive Immunotherapy of Ovarina Cancer Patients—A Pilot Study.

Dr. Freedman first submitted this protocol on March 22, 1993. During the June 7–8, 1993, RAC meeting, this protocol was deferred until the investigators could return to the full RAC with the following information: (1) Data demonstrating efficient transduction of TIL, (2) sufficient information regarding demonstration of selectivity, i.e., specific trafficking of TIL to tumor, (3) complete statistical analysis, (4) revised Informed Consent document in simplified language, and (5) address concerns about patient responsibility for research-related costs. The motion to defer the protocol pending full RAC review of additional information passed by a vote of 18 favor, 0 opposed, and no abstentions.

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Vogelzang

On January 6, 1994, Dr. Nicholas Vogelzang, University of Chicago, Chicago, Illinois, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Immunotherapy for Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions.

VII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Roth

On January 4, 1994, Dr. Jack A. Roth of the MD Anderson Cancer Center, University of Texas, Houston, Texas, resubmitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Clinical Protocol of Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC).

Dr. Roth first submitted this protocol on March 19, 1992. During the September 14–15, 1992, RAC meeting, approval of this protocol was recommended contingent on the review and approval of the following information by RAC primary reviewers (Drs. Miller, Hirano, and Geiduschek): (1) Data demonstrating the transforming potential of 100 milliliters of retroviral supernatant analogous to the preparation that will be used for the clinical protocol, (2) data obtained from *in vitro* mixing experiments, (3) *in vitro* data demonstrating that the new vector preparations have activity, and (4) incorporation of minor changes in the Informed Consent document as noted by Drs. Carmen and Hirano. The motion passed by a vote of 18 in favor, 0 opposed, and no abstentions. On May 11, 1993, Dr. Roth submitted material in response to the RAC's stipulations for approval and a request for the following modifications: (1) The producer cell line will be amended to include GP+*env*AM12, and (2) the clinical protocol grade supernatant will be produced by Microbiological Associates. On June 15, 1993, the primary reviewers agreed to the modification of stipulation, #1, as requested by Dr. Roth. The revised stipulation is: (1) submit data demonstrating the transforming potential of a single patient dose, i.e., 10ml of retrovirus supernatant at 1×10^6 CFU/ml. The primary reviewers did not accept subsequent data submitted by Dr. Roth as adequately fulfilling the stipulations for approval of the protocol. For this reason, the primary reviewers requested that the materials submitted by Dr. Roth should be reviewed by the full RAC at its December 2–3, 1993, meeting. During the December 1993 meeting, the consensus of the RAC was that the protocol was considered administratively inactivated; therefore, RAC approval of the protocol was withdrawn. The RAC recommended that Dr. Roth submit a revised protocol including all additional data for review by the full RAC, based on the following: (1) Failure of the primary reviewers to recommend approval of the protocol, (2) lengthy delays in the presentation of data, (3) the fact that there are several new members who were not on the RAC at the time the original protocol was reviewed, and (4) the proposed use of a new vector. The RAC noted that if Dr. Roth submits a revised protocol for full RAC review, new primary reviewers will be assigned. The RAC recommended that the Office of Recombinant DNA Activities forward a letter to Dr. Roth with recommendations for resubmission of his protocol for full RAC review. The Office of Recombinant DNA Activities forwarded a letter to Dr. Roth on December 21, 1993, requesting submission of a revised protocol by February 4, 1994.

VII. Addition to Appendix D of the NIH Guidelines Regarding Deliberate Transfer of a Chloramphenicol Resistance Gene to an Avirulent Strain of Rickettsia prowzaeki/Dr. Policastro

On January 4, 1994, Dr. Paul Policastro of the National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana, resubmitted a request regarding the deliberate transfer of a gene coding for chloramphenicol resistance to an avirulent strain of Rickettsia prowzaeki.

Dr. Policastro first submitted this request on March 23, 1993. During its June 7–8, 1993, meeting, the RAC deferred approval of this request by a vote of 20 in favor, 0 opposed, and no abstentions. The RAC deferred approval until the investigator submits the following data for full RAC review: (1) Data demonstrating that the construct is safe and useful, and (2) *in vitro* data demonstrating the selective advantage of chloramphenicol resistance over other selectable markers.

IX. Report on Minor Modifications to NIH-Approved Human Gene Transfer Protocols

Dr. LeRoy Walters, RAC Chair, will present an update on minor modifications to NIH-approved human gene transfer protocols.

X. Amendments to Footnotes 21 and 22 and Section III–A–3 of the NIH Guidelines Regarding Recombinant DNA Vaccines

Dr. Leonard Post, Chair of the Working Group on Vaccines, will present an overview of the proposed amendments to Footnotes 21 and 22. The proposed amendments will define those categories of experiments involving the administration of recombinant DNA vaccines that are exempt from RAC review and National Institutes of Health and Institutional Biosafety Committee approval.

XI. Amendments to Sections I, III, IV, and V of the NIH Guidelines and the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider) Regarding NIH (ORDA) Review and Approval of Certain Categories of Human Gene Transfer Experiments That Qualify for the Accelerated Review Process

Dr. Robertson Parkman, Chair of the Working Group on Accelerated Review Protocols, will present an overview of the proposed amendments to the NIH Guidelines and the Points to Consider. The proposed amendments will: (1) Establish an accelerated review process for certain categories of human gene transfer experiments (i.e., "umbrella" multiple site protocols in which the Principal Investigator is responsible for the quality control and data reporting for research conducted at all sites, and duplicate protocols conducted at sites other than those originally approved by the RAC and in which there is a new Principal Investigator), (2) allow the National Institutes of Health (Office of Recombinant DNA Activities) to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with the NIH Guidelines, (3) allow the National Institutes of Health (Office of Recombinant DNA Activities) to approve those categories of human gene transfer experiments that qualify for the accelerated review process in consultation with the Chair and one or more RAC members, as necessary, and (4) exempt certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects which are not covered by Footnote 21. All human gene transfer experiments approved by the National Institutes of Health (Office of Recombinant DNA Activities) through the accelerated review process will be provided in a report by the RAC Chair at the next regularly RAC meeting and will be included in the list of approved experiments which is available from the Office of Recombinant DNA Activities, Building 31, room 4B11, Bethesda, Maryland 20892. The RAC recommends that these amendments be published in the Federal Register for public comment and review by the full RAC during its March 2–3, 1994, meeting. The proposed amendments to the NIH Guidelines are as follows:

"Section I. Scope of the NIH Guidelines"

"Section I–A. Purpose"

[No Changes] "The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) recombinant DNA molecules, and (ii) organisms and viruses containing recombinant DNA molecules."

[No Changes] "Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, are obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH, or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval."

[Amended] "Certain experiments that involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Footnote 21) shall be considered Major Actions to the NIH Guidelines (see Section IV–C–1–b–(1)–(d)), and shall require RAC review and NIH Director approval, if determined by NIH (ORDA) in consultation with the RAC Chair and/or one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review (see Section III–A–3)."

[Addition] "Experiments involving the transfer of recombinant DNA to one or more human subjects that are not considered under Section III–A–3 may qualify for Accelerated Review (see Section III–B–2 of the NIH Guidelines and Part V of the Points to Consider) and will be considered as Minor Actions to the NIH Guidelines (see Section IV–C–1–b–(2)–(a)). Actions that qualify for Accelerated Review (see Section III–B–2) will be reviewed and approved by NIH (ORDA) in consultation with the RAC Chair and/or one or more RAC members, as necessary."

[Addition] "Certain experiments involving the transfer of recombinant DNA into one or more human subjects (see Footnote 21) may be considered exempt from RAC and/or NIH (ORDA) review and/or NIH Director approval and only require registration with NIH (ORDA) (see Section III–C–6)."

[No Changes—Section I–B through II.]

"Section III. Experiments Covered by the NIH Guidelines"

[Amended] "* * * Any change in containment level, which is different from those specified in the NIH Guidelines may not be initiated without the express approval of NIH (ORDA) (see Minor Actions, Section IV–C–1–b–(2) and its subsections.)"

"Section III–A. Experiments that Require RAC Review and NIH and IBC Approval Before Initiation"

[Amended] "Experiments in this category are considered Major Actions to the NIH Guidelines (see Section IV–C–1–b–(1)) and cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the Federal Register for 15 days of comment, review by the RAC, and specific approval by NIH (not applicable for Expedited Review single patient human gene transfer experiments considered under Part VI of the Points to Consider). The containment conditions for such experiments will be recommended by the RAC and set by NIH at the time of approval. Such experiments also require IBC approval before initiation. Specific experiments already approved in this section may be obtained from ORDA, NIH, Building 31, room 4B11, Bethesda, Maryland 20892."

[No Changes—Section III–A–1 through III–A–2].

[Amended] "Section III–A–3. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Footnote 21) shall be considered Major Actions (see Section IV–C–1–b–(1)–(d)), and shall require RAC review and NIH Director approval, if determined by NIH (ORDA), in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree to risk to human health or the environment, or (iii) contain information determined to require further public review. The requirement for RAC review should not be considered to preempt any other required review of experiments with one or more human subjects. Relevant IBC and Institutional Review Board (IRB) reviews of the proposal should be completed before submission to NIH. See Part III–A of the Points to Consider for guidelines for submission of human gene transfer protocols. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects may qualify for the Accelerated Review process (see Section III–B–2). Certain categories of experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and that are not covered by Footnote 21, may be considered exempt from RAC and/or NIH (ORDA) review and/or NIH Director approval and only require registration with NIH (ORDA) (see Section III–C–6)."

[No Changes—Section III–B through III–B–1(1).]

[Addition] "Section III–B–2. Human Gene Transfer Experiments that Qualify for Accelerated Review and Approval by NIH (ORDA)"

[Addition] "As determined by the NIH (ORDA), in consultation with the RAC Chair and one or more RAC members, as necessary, certain categories of human gene transfer experiments may be considered as Minor Actions to the NIH Guidelines and qualify for accelerated review and approval (see Section IV–C–1–b–(2)(a)). The RAC Chair will present a report of all NIH (ORDA)-approved human gene transfer protocols at the next regularly scheduled RAC meeting. If NIH (ORDA) determines that an experiment does not qualify for the accelerated review

process, the PI must submit the proposal for full RAC review at least 8 weeks prior to the next scheduled RAC meeting (see Section III–A–3). See Part III–A of the Points to Consider for guidelines for submission of human gene transfer protocols."

[Addition] "Section III–B–3. Minor Modifications to Human Gene Transfer Experiments."

[Addition] "A minor change in a human gene transfer protocol is a change that does not significantly alter the basic design of the protocol and that does not increase risk to human subjects or the environment. NIH (ORDA) will consider the change, in consultation with the RAC Chair and one or more RAC members, as necessary, after approval has been obtained by the relevant IRB and IBC. The RAC Chair will provide a report on any such approvals at the next regularly scheduled RAC meeting."

[No Changes—Section III–C through III–C–6.]

[Addition] "Section III–C–6. Human Gene Transfer Experiments not Covered by Footnote 21."

[Addition] "Experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, and that are not covered by Footnote 21, must be registered with NIH (ORDA). The relevant IBC and IRB must review all experiments in this category prior to their initiation. For experiments in this category, the registration document must include:"

[Note: The RAC will discuss the information that should be filed with ORDA for experiments in this category.]

[No Changes—Section III–D through IV–C–1B.]

"Section IV–C–1–b–(1), Major Actions"

[Amended] "To execute major actions, the NIH Director must seek the advice of the RAC and provide an opportunity for pubic and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions shall be published in the **Federal Register** at least 15 days before the meeting, and the NIH Director shall also publish the proposed actions in the **Federal Register** for comment at least 15 days before the meeting (not applicable for Expedited Review human gene transfer experiments considered under Part VI of the Points to Consider. In addition, the NIH Director's proposed decision, at his/her discretion, may be published in the **Federal Register** for 15 days of comment before final action is taken. The RAC and IBC Chairs shall be notified of the following decisions:"

[No changes—Section IV–C–1–b–(1)–(a) through IV–C–1–b–(1)–(f).]

[Amended] "Section IV–C–1–b–(2). Minor Actions"

[Amended] "NIH (ORDA) shall carry out certain functions as delegated to it by the NIH Director (see Section IV–C–3). Minor actions, as determined by NIH (ORDA) in consultation with the RAC Chair and one or more RAC members, as necessary, will be transmitted to the RAC and IBC Chairs:"

[Delete the current Section IV–C–1–b–(2)–(a). Interpreting and determining containment levels upon request by ORDA;]

[Addition] "Section IV–C–1–b–(2)–(a). Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that qualify for the Accelerated Review process (see Section III–B–2);"

[Addition] "Section IV–C–b–(2)–(b). Reviewing and approving minor changes to human gene transfer protocols approved under Section III–A–3 and III–B–2;"

[Renumbered] "Section IV–C–1–b–(2)–(c). Changing containment levels for experiments that are specified in the NIH Guidelines (see Section III);"

[Renumbered] "Section IV–C–1–b–(2)–(d). Assigning containment levels for experiments not explicitly considered in the NIH Guidelines;"

[Renumbered] "Section IV–C–1–b–(2)–(e). Revising the Classification of Etiologic Agents for the purpose of these NIH Guidelines (See Footnote 1)."

[Delete Section IV–C–1–b–(3). Other Actions. The NIH Director's decision will be transmitted to the RAC and IBC Chairs:]

[Renumbered] "Section IV–C–1–b–(2)–(f). Interpreting the NIH Guidelines for experiments to which the NIH Guidelines specifically assign containment levels;"

[Renumbered] "Section IV–C–1–b–(2)–(g). Setting containment under Section III–C–1–d and Section III–C–3–d;"

[Renumbered] "Section IV–C–1–b–(2)–(h). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I–II);"

[Renumbered] "Section IV–C–1–b–(2)–(i). Decertifying already certified host-vector systems;"

[Renumbered] "Section IV–C–1–b–(2)–(j). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F);"

[Renumbered/Amended] "Section IV–C–1–b–(2)–(k). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–C–1–b–(2)–(d).]

[Renumbered] "Section IV–C–1–b–(4). The Director, NIH, shall conduct, support, and assist training programs in laboratory safety for IBC members, BSOs, PIs, and laboratory staff."

[Amended] "Section IV–C–2. Recombinant DNA Advisory Committee (RAC) * * * The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–C–1–b–(1)."

[Amended] "Section IV–C–3. The Office of Recombinant DNA Activities (ORDA). The ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including Institutions, BSOs, PIs, Federal agencies, state and local governments, and institutions in the private sector. The ORDA shall carry out such other functions as may be delegated to it by the Director, NIH including those authorities described in Section IV–C–1–b–(2). In addition, ORDA shall be responsible for the following."

[No Changes—Section IV–C–3–a.]

[Addition] "Section IV–C–3–b. Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, in consultation with the RAC Chair and one or more RAC members, as necessary, that qualify for the Accelerated Review process (see Section III–B–2);"

[Addition] "Section IV–C–3–c. Reviewing and approving minor changes to human gene transfer protocols approved under Sections III–A–3 and III–B–2, in consultation with the RAC Chair and one or more RAC members, as necessary;"

[Renumbered] "Section IV–C–3–d. Reviewing and approving IBC membership;"

[Renumbered] "Section IV–C–3–e. Publishing in the **Federal Register**:"

[Renumbered] "Section IV–C–3–e–(1). Announcements of RAC meetings and agendas at least 15 in advance;"

[No Changes] "NOTE—If the agenda for a RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least seventy-two hours in advance of the meeting."

[Renumbered] "Section IV–C–3–e–(2). Proposed major actions of the type falling under Section IV–C–1–b–(1) at least 15 days prior to the RAC meeting at which they will be considered; and"

[Delete old Section IV–C–3–c–(3). The NIH Director's final decision on recommendations made by the RAC.]

[Renumbered/Amended] "Section IV–C–3–f. Serve as the focal point for data management of NIH-approved human gene transfer protocols approved under Section III–A–3 and III–B–2 and registered with NIH (ORDA) as required under Section III–C–6."

[Renumbered] "Section IV–C–3–g. Serve as executive secretary of the RAC."

[Addition] "Section IV–C–3–h. Maintain a list of major and minor actions to the NIH Guidelines approved under Sections III–A–3 and III–B–2 and a list of experiments registered with NIH (ORDA) as described in Section III–C–6."

[No Changes—Section IV–C–4 through V–T].

[The RAC Will Consider Revision of this Footnote.] "Footnote 21. Sections III–A–3 and III–B–2 cover only those experiments in which the intent is to modify stably the genome of cells of one or more human subjects. Other experiments involving the deliberate transfer of recombinant DNA into one or more human subjects such as feeding of bacteria containing recombinant DNA or the administration of vaccines containing recombinant DNA are not covered in Sections III–A–3 and III–B–2."

[The RAC may Modify this Footnote According to Footnote 21 Revision] "Footnote 22. For recombinant DNA experiments in which the intent is to modify stably the genome of cells of one or more human subjects (see Sections III–A–3 and III–B–2)."

[No Changes—Section VI.]

The proposed amendments to the Points to Consider are as follows:

[Amended] "These Points to Consider apply to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH. Researchers not covered by the NIH

Guidelines are encouraged to use the Points to Consider. Experiments in which recombinant DNA is introduced into one or more human subjects with the intent of stably modifying the subject's genome are covered by Sections III–A–3 and III–B–2 of the NIH Guidelines (see Footnote 21 of the NIH Guidelines). Experiments in which recombinant DNA is introduced into one or more human subjects with no risk of stably modifying the subject's genome are covered under Section III–C–6 of the NIH Guidelines (see Footnote 21 of the NIH Guidelines). Sections III–A–3, III–B–2, and III–C–6 of the NIH Guidelines apply both to recombinant DNA and to DNA or RNA derived from recombinant DNA."

[Amended] "This document is intended to provide guidance in preparing proposals for NIH consideration under Sections III–A–3 and III–B–2 of the NIH Guidelines. Section III–A–3 addresses Major Actions to the NIH Guidelines involving the transfer of recombinant DNA into one or more human subjects that have been determined by NIH (ORDA), in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. Proposals considered under Section III–A–3 of the NIH Guidelines will be reviewed by the RAC and approved by the NIH Director. RAC review of experiments considered under Section III–A–3 of the NIH Guidelines will follow publication of a precis of the proposal in the Federal Register and an opportunity for public comment. Section III–B–2 addresses Minor Actions to the NIH Guidelines involving the transfer of recombinant DNA into one more human subjects that have been determined by NIH (ORDA), in consultation with the RAC Chair and one or more RAC members, as necessary, to qualify for the accelerated review process. Proposals considered under Sections III–A–3 and III–B–2 will be on a case-by-case basis. A list of actions to the NIH Guidelines approved under Sections III–A–3 and III–B–2 involving the transfer of recombinant DNA into one or more human subjects is available from the Office of Recombinant DNA Activities, Building 31, Room 4B11, National Institutes of Health, Bethesda, Maryland, 20892. The list of actions to the NIH Guidelines involving the transfer of recombinant DNA into one or more human subjects does not include experiments considered to be exempt from RAC and NIH (ORDA) review under Section III–C–6 of the NIH Guidelines."

[No changes] "In general, it is expected that the transfer of recombinant DNA into one or more human subjects will not present a risk to public health or to the environment as the recombinant DNA is expected to be confined to human subjects. Nevertheless, Part I–B–4–b specifically asks the researchers to address this point."

[No changes] "This document will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed."

[Amended] "A proposal involving the transfer of recombinant DNA into one or more human subjects will be considered by the RAC and/or NIH (ORDA) only after the protocol has been approved by the local Institutional Biosafety Committee (IBC) and the local Institutional Review Board (IRB) in accordance with DHHS Federal Regulations for the Protection of Human Subjects (45 Code of Federal Regulations, part 46). (If a proposal involves children, special attention should be paid to subpart D of these DHHS regulations.) The IRB and IBC may, at their discretion, condition their approval on further specific deliberation by the RAC and/or NIH (ORDA). Consideration of human gene transfer proposals by the RAC and/or NIH (ORDA) may proceed simultaneously with review by other involved federal agencies (See Part VI–A) provided that NIH (ORDA) is notified of the simultaneous review. Meetings of the full RAC will be open to the public except where trade secrets or proprietary information would be disclosed. The committee prefers that proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. The public review of these protocols will serve to inform the public not only on the technical aspects of the proposals but also on the meaning and significance of the research."

[No changes] "The clinical application of recombinant DNA techniques raises two general kinds of questions: (i) The questions usually discussed by IRBs in their review of any proposed research involving one or more human subjects; and (ii) broader issues. The first type of question is addressed principally in Part III of this document. Several broader issues are discussed later in this Introduction and in part II below."

[Amended] "Following this Introduction, this document is divided into four parts. Part I requests a description of the protocol with special attention to the short-term risks and benefits of the proposed research to the patient and to other people, the selection of patients, informed consent, and privacy and confidentiality. In part II, investigators are requested to address special issues pertaining to the free flow of information about the clinical trials. These issues lie outside the usual purview of IRBs and reflect general public concerns about biomedical research. Part III summarizes other requested documentation that will assist the RAC and/or NIH (ORDA) in its review of the proposals. Part IV specifies reporting requirements."

[Amended] "The RAC will not at present entertain proposals for germ-line alterations but will consider for approval protocols involving somatic cell gene transfer. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning gene into a patient's somatic cells. In germ line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring."

[No changes] "The acceptability of human somatic cell gene therapy has been addressed in several public documents as well as in numerous academic studies. The 1982 report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, Splicing Life, resulted from a two-year process of public deliberations and hearings. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, Human Gene Therapy, which concluded: civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies. In light of this public support, the RAC is prepared to consider proposals for somatic cell gene therapy."

[Amended] "In its evaluation of proposals involving the transfer of recombinant DNA into one or more human subjects, the RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigations, namely, to protect the health and well-being of the individual subject(s) being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) Vertical transmission of genetic changes from an individual to his of her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and/or NIH (ORDA) to assess the possibility that the proposed experiments will inadvertently affect reproductive cells or lead to infection of other people (e.g., personnel or relatives)."

[Amended] "In recognition of the social concern that surrounds the subject of gene transfer, the RAC and NIH (ORDA) will cooperate with other groups in assessing the possible long-term consequences of the transfer of recombinant DNA into one or more human subjects and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology."

[No changes] "Responses to the questions raised in these Points to Consider should be provided in the form of either written answers or references to specific sections of the protocol or its appendices."

[No changes] "Investigators should indicate points which are not applicable with a brief explanation. Investigators submitting proposals that employ essentially the same vector systems (or with minor variations), and/or that are based on the same preclinical testing as proposals previously reviewed by the RAC, may refer to preceding documents without having to rewrite such material."

[No Changes—Part I–A through I–C.]

[Proposed Changes—Parts I–D and I–E. See agenda item XII proposed amendments.]

[No Changes—Parts II through II–B.]

[Delete—Part III. Requested Documentation. In addition to responses to the questions raised in these Points to Consider, please submit the following materials:]

[Delete—Part III–A. Your protocol as approved by your local IRB and IBC.]

[Delete—Part III–B. Results of local IRB and IBC deliberations and recommendations that pertain to your protocol.]

[Delete—Part III–C. A one-page scientific abstract of the protocol.]

[Delete—Part III–D. A one-page description of the proposed experiment in nontechnical language.]

[Delete—Part III–E. Curricula vitae for key professional personnel.]

[Delete—Part III–F. An indication of other federal agencies to which the protocol is being submitted for review.]

[Delete—Part III–G. Any other material which you believe will aid in the review.]

[Renumbered/Amended] "Part III. Guidelines for the Submission of Human Gene Transfer Protocols"

[Addition] "For consideration of human gene transfer protocols under Section III–A–3 and III–B–2 of the NIH Guidelines the following criteria will apply:"

[Renumbered] "Part III–A. Investigator-Submitted Material"

[Renumbered/Amended] "Part III–A–1. Written proposals must be submitted in the following order: (1) Scientific abstract—1 page; (2) non-technical abstract—1 page; (3) Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) approvals and their deliberations pertaining to your protocol; (4) Points to Consider (Parts I and II)—5 pages; (5) protocol (as approved by the local IRB and IBC)—20 pages excluding appendices; (7) Informed Consent Document—approved by the IRB; (8) appendices including tables, figures, and manuscripts; (9) Curricula vitae—2 pages in Biographical sketch format; and (10) an indication of other Federal agencies to which the protocol is being submitted for review."

[Renumbered] "Part III–A–2. When a proposal has been submitted previously, there should be a short section (≤ 200 words) immediately following the abstracts that summarizes the major revisions since the last review."

[Renumbered] "Part III–A–3. Data provided must include: (i) A description of the elements in the vector, (ii) the source of that information, (iii) the method by which sequence data were compiled, and (iv) three 3½ inch diskettes with the vector sequence in ASCII format."

[Renumbered] "Part III–A–4. Time Frame for Submissions."

[Added] "Note: Time frames are applicable only to protocols that are determined by NIH (ORDA) to require full RAC review and NIH Director approval. Times frames do not apply to Accelerated Review human gene transfer experiments (see Section III–B–2 of the NIH Guidelines) or those that only require registration with NIH (ORDA) (see Section III–C–6 of the NIH Guidelines)."

[Renumbered] "Part III–A–4–a. Written material from PI must be submitted at least 8 weeks before the RAC meeting at which it will be reviewed."

[Renumbered] "Part III–A–4–b. Written comments from the primary reviewers to the PI must be submitted at least 4 weeks before the RAC meeting at which it will be reviewed."

[Renumbered] "Part III–A–4–c. Written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to ORDA at least 2 weeks before the RAC meeting."

[Renumbered] "Part III–A–5. Oral Responses to the RAC."

[No changes] "Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted at least 2 weeks prior to the RAC meeting is prohibited."

[Renumbered] "Part III–B. Primary Reviewers' Responses."

[Renumbered] "Part III–B–1. Primary Reviewers' Written Comments."

[No changes] "The primary reviewers' written comments on a proposal should include the following:"

[Renumbered] "Part III–B–1–a. Emphasize the issues related to gene marking, gene transfer, or gene therapy."

[Renumbered] "Part III–B–1–b. State explicitly whether the Points to Consider have been addressed satisfactorily."

[Renumbered] "Part III–B–1–c. Examine the scientific rationale, scientific context (relative to other proposals reviewed by the RAC), whether the preliminary *in vitro* and *in vivo* data were obtained in appropriate models and are sufficient, and whether questions related to safety, efficacy, and social and ethical context have been resolved."

[Renumbered] "Part III–B–1–d. Whenever possible, criticisms of Informed Consent documents should include suggested revisions for the RAC to consider, provided as written alternatives."

[Renumbered] "Part III–B–1–e. Primary reviews should also state whether the proposal is: (i) Acceptable as written, (ii) expected to be acceptable with specific revisions or after satisfactory responses to specific questions raised on review, or (iii) unacceptable in its present form."

[Renumbered] "Part III–B–2. Oral Discussions by Primary Reviewers at the RAC Meeting."

[Renumbered] "Part III–B–2–a. It should be possible for most primary reviewers to present their oral reviews in 5 minutes or less."

[No changes—Part VI through VI–B.]

[Delete—Part V. Minor Modifications to Human Gene Transfer Experiments Approved by the RAC and NIH Director Under Section III–A–3 of the NIH Guidelines. A minor modification in a protocol approved by the RAC and NIH Director under Section III–A–3 (see Section IV–C–1–b–1—Major Actions) is a change that does not significantly alter the basic design of a protocol and that does not increase risk to the subject(s). If the change has been approved by the relevant IRB and IBC, then the Chair of the RAC may give approval. It is expected that the Chair will consult with one or more members of the committees, as necessary. The RAC Chair will report on any such approvals at the next regularly scheduled RAC meeting.]

[Addition] "Part V. Procedures to be Followed for Accelerated Review of Human Gene Transfer Experiments by NIH (ORDA) Under Section III–B–2 of the NIH Guidelines."

[Addition] "Part V–A. Human gene transfer experiments in this category must be in accordance with the provisions of Section III–B–2 of the NIH Guidelines. If the human gene transfer protocol does not qualify for Accelerated Review (see Section III–B–2 of the NIH Guidelines) as determined by NIH (ORDA), then the PI must submit the experiment for full RAC review and NIH approval in accordance with Section III–A–3 of the NIH Guidelines."

[Addition] "Part V–B. No protocol shall be considered without IBC and IRB approval."

[Addition] "Part V–C. At this time, all gene transfer protocols must be considered experimental."

[Addition] "Part V–D–1. PIs requesting Accelerated Review (see Section III–B–2 of the NIH Guidelines), must submit the relevant documentation in accordance with Part III of the Points to Consider. NIH (ORDA) will notify the investigator whether the proposed study qualifies for the Accelerated Review process. If NIH (ORDA) determines that an experiment does not qualify for the Accelerated Review process the PI must submit the proposal for full RAC review at least 8 weeks prior to the next scheduled RAC meeting."

[Addition] "Part V–E. It is expected that NIH (ORDA) will consult with the RAC Chair and one or more RAC members, as necessary, when considered Accelerated Review human gene transfer protocols (see Section III–B–2 of the NIH Guidelines—."

[Addition] "Part V–F. The Chair of the RAC will provide a report on all human gene transfer protocols that have been approved by NIH (ORDA) at the next regularly scheduled RAC meeting."

[Addition] "Part V–F–1. In accordance with Part VI, Reporting Requirements, of the Points to Consider, any adverse effects of the treatments should be reported in writing immediately to both the local IRB and the NIH Office for Protection from Research Risks, and the report should also be forwarded to NIH (ORDA)."

[Addition] "Part V–F–2. In accordance with Part IV, Reporting Requirements, of the Points to Consider, reports regarding the general progress of patients should be filed with both the local IRB and ORDA within six months of the commencement of the experiments and at six-month intervals. In the event of a patient's death, a summary of the special post-mortem studies and statement of the cause of death should be submitted to the IRB and ORDA, if available."

[Amended] "Part VI. Procedures to be Followed for Expedited RAC Review and NIH Approval of Single-Patient Human Gene Transfer Protocols Considered Under Section III–A–3 (see Section IV–C–1–b–(1)–(d) of the NIH Guidelines)."

[Amended] "Part VI–A. An investigator submitting a request to NIH (ORDA) for

expedited review of a single patient gene transfer protocol must provide detailed information regarding the necessity of expedited review."

[No changes—Part VI–B through VI–F.]

[Amend] "Part VI–G. The NIH (ORDA) will report to the RAC following expedited review and will include all of the materials on which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained."

[No changes—Part VI–H through VI–J.]

[Renumber "Part VII. Footnotes."

[No changes] "The Food and Drug Administration (FDA) has jurisdiction over drug products intended for use in clinical trials of human gene transfer. For general information on FDA's policies and regulatory requirements, (see the **Federal Register**, Volume 51, pages 23309–23313, 1986)."

[No changes] "The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

XII. Amendments to Part I–D of the Points To Consider, NIH Guidelines, Regarding Informed Consent

During the December 2–3, 1993, meeting, Dr. Gary Ellis, Director of the Office for Protection from Research Risks (OPRR), NIH, Bethesda, Maryland, responded to the written comments submitted by Dr. Zallen, Chair of the Working Group on Informed Consent Issues. Dr. Ellis noted the RAC's concern regarding specific issues that should be addressed in human gene transfer protocol Informed Consent documents, i.e., request for autopsy, recommendations for male, female contraception, separate Informed Consent documents when gene therapy is separate from a clinical protocol, commitment to long-term patient follow-up, and financial responsibility of the institution for all research-related costs. During his presentation, Dr. Ellis provided the RAC with background information regarding the roles of both OPRR and local Institutional Review Boards (IRB) in the review of research proposals involving human subjects. Dr. Ellis recommended that the RAC draft a letter outlining their specific recommendations to OPRR for distribution and consideration by the local IRBs.

In a memorandum dated December 23, 1993, Dr. Ellis further clarified the avenues that should be pursued by the RAC with regard to the "quality and content of informed consent documents into constructive changes in the informed consent process," specifically in relation to human gene transfer. Dr. Ellis recommended that the Points to Consider should be amended to introduce consistency in the Informed Consent document language; therefore, Part I–D of the Points to Consider has been expanded. Proposed Part I–D–1 addresses the informed consent process. Proposed Part I–D–2 addresses the informed consent document. Part I–E has been incorporated into I–D–1. The proposed amendments read:

"Part I–D–1. Informed Consent Process

"In accordance with the requirements of DHHS regulations for the protection of human subjects (45 CFR, part 46), investigators shall indicate how patients will be informed about the proposed study and how their consent will be solicited. If the study involves pediatric or mentally handicapped patients, describe the procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient.

"Part I–D–1–a. Communication of the Study to Potential Participants

"Part 1–D–1–a–(1). What processes or procedures will be used to identify individuals who might be candidates for the proposed study?

"Part 1–D–1–a–(2). Which members of the research group and/or the institution will be involved in contacting potential participants and in describing the study to them? Where will discussions or other means of informing such individuals about the proposed study take place? What is the length of time that potential participants will have to make a decision about their participation in the proposed study?

"Part 1–D–1–a–(3). How will the following items be dealt with during the informed consent process:

"Part I–D–1–a–(3)–(a). How will the major points covered in Parts I–A through I–C of the *Points to Consider* be disclosed to potential participants in the proposed study and/or their parents or guardians in language that is understandable to them?

"Part I–D–1–a–(3)–(b). How will the innovative character and the theoretically possible adverse effects of the experiment be discussed with the subjects and/or their parents or guardians? How will the potential adverse effects of the experiment be compared with the consequences of the disease?

"Part I–D–1–a–(3)–(c). How will participants and/or their parents or guardians be informed about the innovative character of the experiment and that their participation in the proposed study may lead to interest by the media?

"Part I–D–1–1–(3)–(d). How will the participants and/or their parents or guardians be informed about the possible irreversible consequences of procedures performed?

"Part I–D–1–a–(3)–(e). How will the participants and/or their guardians be informed about any expectation that they will cooperate in long-term follow-up?

"Part I–D–1–a–(3)–(f). How will the participants and/or their guardians be informed about the adverse medical consequences that may occur if the subjects withdrew from the study once the experiment has started?

"Part I–D–1–a–(3)–(g). How will the participants and/or their parents or guardians be informed that permission to perform an autopsy will be requested in the event of a subject's death? (An autopsy shall be requested due to the need for an accurate determination of the precise cause of the subject's death and the potential knowledge that may be gained from such information.)

"Part I–D–1–a–(3)–(h). How will estimates of any financial costs associated with participation in the experiment and in the long-term follow-up to the experiment that are not covered by the investigators or the institutions involved be provided to potential participants and/or their parents or guardians? How will comparable financial information for other available alternatives, including other investigational therapies, be provided?

"Part I–D–1–1–(1)–(4). If there are more potential candidates than the study can accommodate, how will the decision be made about which to include? How will those who are excluded be informed?

"Part I–D–1–b. Privacy and Confidentiality

"Part I–D–1–b–(1). Provide a description of the measures that will be taken to protect the privacy of patients and their families as well as to maintain the confidentiality of the research date.

"Part I–D–1–b–(2). Provide a description of the provisions that will be made to maintain the confidentiality of research data, especially in cases where data could be lined to individual patients.

"Part I–D–1–b–(3). Provide a description of the provision that will be made to honor the wishes of individual subjects and/or the parents or guardians of pediatric or mentally handicapped patients, as to whether, when, or how the identity of patients may be publicly disclosed.

"Part I–D–1–c. Special Issues

"Although the following issues are beyond the normal purview of the local IRBs, the RAC requests that investigators respond to the following questions:

"Part I–D–1–c–(1). Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

"Part I–D–1–c–(2). What steps will be taken to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

"Part I–D–2. Informed Consent Document

"Part I–D–2–a. When gene transfer is a procedure separate from an IRB-approved clinical protocol, separate informed Consent documents shall be submitted for both the gene transfer and clinical protocols.

"Part I–D–2–b. Investigators submitting human gene transfer proposals for RAC review must include the Informed Consent document as approved by the local IRB. This document shall include the following specific information in addition to any requirements of the DHHS regulations for the protection of human subjects (45 CFR, part 46):

"Part I–D–1–b–(1). Purpose of the Study

"For experiments in which there is no therapeutic intent, an explanation shall be

provided that no direct clinical benefit is expected as a result of the subject's participation in the study; however, knowledge may be gained that could benefit others.

"Part I–D–2–b–(2). Description of the Procedures

"The subject will be provided a detailed description of the procedures associated with the proposed study, including a description of the gene transfer procedures in non-technical language.

"Part I–D–2–b–(3). Possible Risks, Discomforts, and Side Effects

"The subject will be provided with a detailed description of the risks, discomforts, and side effects, including a warning about possible unforeseen risks, that may result from their participation in the proposed study.

"Part I–D–2–b–(4). Benefits

"The subject will be provided with a detailed description of the possible benefits of the proposed study.

"Part I–D–2–b–(5). Contact Persons

"The subject will be provided with a list of persons who are available to answer any questions relating to their participation in the proposed study and to contact in the event that questions arise during the course of the study, including the follow-up period.

"Part I–D–2–b–(6). Alternatives

"The subject shall be informed about the availability of other therapies, including the possibility of other investigational therapies.

"Part I–D–2–b–(7). Voluntary Participation

"The subject shall be informed that their participation in the study is voluntary and that failure to participate in the study, or withdrawal of consent, will not incur any penalty or loss of benefits to which the subject is otherwise entitled.

"Part I–D–2–b–(8). Confidentiality

"The subject shall be informed that the institution and investigators will make every effort to provide protection from the media in an effort to protect the participant's privacy.

"Part I–D–2–b–(9). Explanation to Participants of the Specific Requirements of Gene Transfer Research

"Part I–D–2–b–(9)–(a). Female subjects shall be informed that they should not be pregnant nor planning to become pregnant during the course of their participation in the study. Male and female subjects shall be informed that barrier contraception is required during the active phase of their participation in the study.

"Part I–D–2–b–(9)–(b). The subject shall be informed about the extent to which he/she will be responsible for any costs associated with medical treatment required as a direct result of research-related injury.

"Part I–D–2–b–(9)–(c). The PI shall request that the subject keep the laboratory informed of a current address and telephone number.

"Part I–D–2–b–(9)–(d). The subject shall be informed that in the event of death, as a result of accident or illness, an autopsy shall be requested because of the importance of the knowledge that may be gained from such studies.

"Part I–D–2–b–(9)–(e). The subject shall be informed that any significant findings resulting from the proposed study will be made known to them and/or their parent or guardian. This would include new information about the nature of the experimental procedure or the physical reactions experienced by other individuals involved in the study.

"Part I–D–2–b–(9)–(f). The subject shall be informed that representatives of applicable Federal agencies (e.g., the NIH and FDA) and representatives of collaborating institutions will have access to the participant's medical records."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: February 7, 1994.

Daryl A. Chamblee, J.D.,

Acting Deputy Director for Science Policy and Technology Transfer.

[FR Doc. 94–3180 Filed 2–10–94; 8:45 am]

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RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 3-4, 1994

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
March 3-4, 1994

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-seventh meeting at 9:00 a.m. on March 3, 1994, at the National Institutes of Health (NIH), Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Ira H. Carmen, University of Illinois
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, The Foundation of Human Genetics
Mariann Grossman, Hospital of the University of Pennsylvania
Susan S. Hirano, University of Wisconsin
Arno G. Motulsky, University of Washington
Robertson Parkman, Children's Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, Virginia Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).

National Institutes of Health Staff:

Bobbi Bennett, OD
Christine Boenning, NIAID
Carol Bosken, NHLBI
Sandra Bridges, NIAID
Bruce Bunnell, NCHGR

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[1]The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Sarah Carr, OD
Barry Goldspiel, CC
Betsey Herpin, NIAID
Christine Ireland, OD
Susan Jenks, NCI
H. Clifford Lane, NIAID
Becky Lawson, OD
Martha Lawson, NIAID
Sachiko Kajigaya, NHLBI
Dai Katayose, NCI
Masako Kawase, NHLBI
Catherine McKeon, NIDDK
David Nelson, NCHGR
Prem Seth, NCI
Thomas Shih, OD
Robert Walker, NIAID
Debra Wilson, OD
Jim Yang, NCI

Others:

Paul Aebersold, Food and Drug Administration
W. French Anderson, University of Southern California
Jack Barber, Viagene, Inc.
Bridget Binko, Cell Genesys
Kenneth Brigham, Vanderbilt University
Gracia Buffleben, ACT UP/Golden Gate
Angelo Canonico, Vanderbilt University
Jeff Carey, Genetic Therapy, Inc.
Joy Cavagnaro, Food and Drug Administration
Henry Chang, Shared Medical Research Foundation
Lan Chang, Institute of Biomedical Science
Yawen Chiang, Genetic Therapy, Inc.
Jon Conary, Vanderbilt University
Rathin Das, Miles, Inc.
Mitchell Finer, Cell Genesys
Ralph Freedman, M. D. Anderson Cancer Center
George Gray, Vical, Inc.
Joanna Hales, Cell Genesys
Lowell Harmison, Self-Employed
Evan Hersh, University of Arizona
Morio Hibino, Kennedy Institute of Ethics, Georgetown University
Doug Hickman, T. Rowe Price
Dan Hoth, Cell Genesys
Allen Kamer, The Pink Sheet
Catherine Killion, Baxter Healthcare Corporation

Paul Kleinman, Biopharmaceutical Writers Service
Lori Kobayashi, Hood College
Steven Kradjian, Vical, Inc.
Richard Lazar, Cell Genesys
Timothy Lestingi, University of Chicago
Irene Lowe, Scipress News Bureau
Stephen Lupton, Targeted Genetics Corporation
Tamie Malaska, Targeted Genetics Corporation
Tony Marcel, TMC Development
Michael McCaughan, The Pink Sheet
Brian McGuire, Cell Genesys
Janice McTeague, Genzyme Corporation
Bruce Merchant, Viagene, Inc.
James Merritt, Viagene, Inc.
Andra Miller, Food and Drug Administration
Michael Nash, M. D. Anderson Cancer Center
Jim Neidhart, University of New Mexico
Krista Nowell, Hood College
Sheryl Osborne, Viagene, Inc.
Robert Overell, Targeted Genetics Corporation
Lalida Panpradit, Hood College
Anne Petruska, The Blue Sheet
Lisa Piercey, BioWorld
Stephen Pijar, University of Maryland
Dennis Piszkiewicz, Baxter Healthcare Corporation
Chris Platsoucas, Temple University
Raj Puri, Food and Drug Administration
Urban Ramstedt, Virus Research Institute
Judy Randal, The Economist
Janet Ransom, Organon Teknika Corporation
Paul Recer, Associated Press
Rex Rhein, Biotechnology Newswatch
Margo Roberts, Cell Genesys, Inc.
Joseph Rosenblatt, University of California, Los Angeles
Jack Roth, M. D. Anderson Cancer Center
Patricia Ryan, Genetic Therapy, Inc.
Masahiko Sato, Taisho University
Bruce Schackman, CIT Group
Hans Schreier, Vanderbilt University
Robert Seeger, Children's Hospital of Los Angeles
G. Terry Sharrer, National Museum of American History
Sharon Smith, Hood College
Lisa Song, Hood College
Arlene Stecenko, Vanderbilt University
Margi Stuart, Prevention Research Center
Frank Sturtz, Progenitor, Inc.

Ruth Suchodolski, Genzyme Corporation
Nevin Summers, Ingenex, Inc.
Anthony Taylor, Gene Therapy Advisory Committee Secretariat of the United Kingdom
Larry Thompson, Medical News Network
Paul Tolstoshev, Genetic Therapy, Inc.
Nicholas Vogelzang, University of Chicago
Katharine Whartenby, Food and Drug Administration
Teruhiko Yoshida, National Cancer Center Research Institute
Krisztina Zsebo, Cell Genesys, Inc.

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that the notice of the meeting was published in the *Federal Register* on February 11, 1994 (58 FR 6702), as required by the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the proposal, followed by responses from the principal investigators. The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters requested that the RAC observe a moment of silence in memory of Dr. Brigid Leventhal who died on February 6, 1994. During her service on the RAC, Dr. Leventhal was instrumental in establishing the current process of data management for all RAC approved human gene transfer protocols. Prior to her appointment to the RAC, Dr. Leventhal served on the RAC Human Gene Therapy Subcommittee on which she reviewed the first human gene transfer experiment approved in the United States. Apart from her inexhaustible dedication to the RAC, Dr. Leventhal is recognized for her major contribution to the development of treatments for pediatric leukemia. The RAC is honored to have had the opportunity to interact with a noted physician and patient advocate such as Dr. Leventhal.

Dr. Walters noted several issues previously identified by the RAC, which were followed up by the Office of Recombinant DNA Activities (ORDA). On December 16, 1993, ORDA sent a memorandum to the NIH Office of Legislative Policy Analysis requesting that the language regarding provision of medical care to subjects injured in the course of their participation in clinical research be followed in the proposed health care reform benefits package. ORDA requested immediate notification of any changes to the proposed language by the Administration or Congress.

In a letter dated January 27, 1994, the Institutional Review Board (IRB) of the University of Alabama, Birmingham, Alabama, responded to the RAC's recommendations regarding revisions to the Informed Consent document of Dr. Eric Sorscher (Protocol #9312-066). The IRB noted that in keeping with the institutional policy, the language regarding research related injury will not be modified.

In a letter dated February 3, 1994, the Human Subjects Review Board of the University of California at San Diego, California, deferred to the RAC's recommendation regarding azidothymidine (AZT) administration in human immunodeficiency virus (HIV) seropositive patients (Dr. Flossie Wong-Staal's Protocol #9309-057). AZT will no longer be required as a safeguard against the possible emergence of replication-competent retroviruses (RCR). However, the Board will maintain its requirement for animal toxicity testing for the first 3 lots of transduced cells produced by the investigators.

In a letter dated February 11, 1994, Viagene, Inc., San Diego, California, notified the

RAC that suggested revisions to Dr. Richard Haubrich's Informed Consent document (Protocol #9312-062) were incorporated and approved by the IRB of the University of California at San Diego.

No response was received to a December 9, 1993, letter to the IRB of the University of Iowa College of Medicine, Iowa City, Iowa, regarding suggested revisions to Dr. Michael Welsh's Informed Consent document (Protocol #9312-067).

At the request of the NIH Office for the Protection from Research Risk, Dr. Walters will address the Federal Coordinating Council for Science, Engineering and Technology, Human Subjects Review Committee regarding the issue of provision of medical care to patients injured in the course of their participation in biomedical research and compensation for research related injury. Dr. Walters noted that in 1976 the University of Washington, Seattle, Washington, adopted a self-insurance program for normal volunteers who participate in "non-clinical research." Dr. Parkman inquired whether a Phase I study would be encompassed as "non-clinical research." Dr. Walters responded that subjects injured in a Phase I study would not be covered. There have been a total of 21 claims since 1976 with the largest claim being $6,000. Dr. Walters said that in the United Kingdom, compensation for research related injuries is not a problem since most of the costs are covered by the National Health System. Dr. Walters will provide an update on this issue at the next RAC meeting.

Dr. Parkman remarked that the compensation issue should be addressed in terms of all clinical research not just for human gene therapy. Dr. Wivel added that inclusion of the language in the proposed health care reform benefits package will be followed up through the NIH Office of Legislative Policy Analysis. Dr. Motulsky said that a bipolar system might evolve since the industry sponsored research is more likely to provide the coverage for medical costs of research injuries than research sponsored by the universities. Dr. Walters said that he has noticed a similar trend in the United Kingdom.

II. RAC WORKING GROUP REPORT - DATA MANAGEMENT/DR. SMITH

Dr. Brian Smith, Chair of the RAC Working Group on Data Management, provided a summary of the responses that were submitted by Principal Investigators in response to ORDA's December 27, 1993, request for additional information. He noted that subsequent information provided by the Principal Investigators adequately addressed the concerns of the RAC. However, Dr. Smith noticed a few problems in the data reporting. In his written comments, Dr. Deisseroth (Protocol #9105-007) questioned the RAC's classification of failure to engraft following bone marrow transplant as an adverse effect. Dr. Smith said that failure to engraft should be considered an adverse effect if the frequency of occurrence is not within the statistical range of the expected outcome. Dr. Smith asked whether the RAC has approved the change of vector in Dr. Rosenberg's study (Protocol #9007-003).

Dr. Smith said that responses were not received from either Dr. Cornetta (Protocol #9202-014) or Drs. Galpin and Casciato (Protocol #9306-048). Dr. Parkman said that

semi-annual data reporting of any possible adverse events are mandated by the *Points to Consider for Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider).*

A motion was made by Dr. Parkman and seconded by Dr. Dronamraju to send follow-up requests for information to Dr. Cornetta and Drs. Galpin and Casciato. If responses are not received by the June 9-10, 1994, RAC meeting, the RAC will reconsider approval of these protocols. The motion passed by a vote of 12 in favor, 1 opposed, and no abstention.

Dr. Walters presented an updated list of approved human gene transfer protocols. The RAC has recommended approval of 67 human gene transfer protocols to date, 59 of these studies have been subsequently approved by the NIH Director, and 8 protocols are contingently approved pending the submission of additional data.

III. DECEMBER 2-3, 1993, RAC MINUTES

The RAC approved a motion made by Dr. Zallen and seconded by Dr. DeLeon to accept the December 2-3, 1993, RAC minutes with the inclusion of minor changes suggested by Drs. Chase, Zallen, and Hirano, by a vote of 13 in favor, 0 opposed, and no abstentions.

IV. CHAIR REPORT ON MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that 11 minor modifications were approved to the following human gene transfer protocols since the December 2-3, 1993, RAC meeting (Attachment II):

DATE	PROTOCOL#	INVESTIGATOR
1/6/94	9206-023	Cynthia Dunbar
1/6/94	9206-024	Cynthia Dunbar
1/6/94	9206-025	Cynthia Dunbar
2/4/94	9212-034	Ronald Crystal
2/17/94	9007-003	Steven Rosenberg
2/17/94	9303-042	Richard Boucher/Michael Knowles
2/17/94	9312-067	Michael Welsh
2/25/94	9306-044	Albert Deisseroth
2/25/94	9306-044	Albert Deisseroth
2/25/94	9209-027	Friedrich Schuening
2/25/94	9209-028	Friedrich Schuening

Responding to the question raised by Dr. Smith during his report concerning the vector

change in Dr. Rosenberg's study (Protocol #9007-003), Dr. Walters said that the minor modification was approved by the ORDA on February 17, 1994.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I STUDY OF IMMUNIZATION WITH GAMMA INTERFERON TRANSDUCED NEUROBLASTOMA CELLS*/DRS. ROSENBLATT AND SEEGER

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. Joseph Rosenblatt of the University of California, Los Angeles, California, and Dr. Robert Seeger of Children's Hospital, Los Angeles, California. Dr. Smith explained that neuroblastoma is the most common extracranial solid tumor in children. Autologous or allogeneic neuroblastoma cell lines will be transduced with the retroviral vector, PHU-γ-IFN, that expresses gamma (γ) interferon. The transduced cells will be lethally irradiated and injected subcutaneously into patients with the objective of inducing an enhanced antitumor response. Both the retroviral vector, pHU-γ-IFN, and the packaging cell line, VCHU, were previously approved by the RAC for Dr. Seigler's melanoma study (Protocol #9306-043). A total of 18 patients under 21 years of age will be divided into two groups: (1) those demonstrating no evidence of disease but who are at significant risk for recurrence or who demonstrate minimal residual disease following the standard chemotherapy and autologous bone marrow transplant regimen; and (2) those who demonstrate persistent or progressive disease. Three dose levels for injections are defined, and 3 patients from each clinical subgroup will be entered at each dose level. A detailed "stop rule" is defined for the trial. The study will characterize safety, toxicity, and clinical antitumor responses. Autologous neuroblastoma cells will be transduced if possible; however, if autologous cells are unavailable, allogeneic cells will be used that have a single human leukocyte antigen (HLA) haplotype match.

Dr. Smith stated that the investigators have provided satisfactory responses to the questions raised in the primary written review of this protocol. The investigators have provided the following subsequent information: (1) data demonstrating that the transduced neuroblastoma cells have been lethally irradiated, (2) data demonstrating that lethally irradiated transduced cells continue to express adequate levels of IFN-γ, (3) data demonstrating a 50% success rate in establishing primary neuroblastoma cell lines, and (4) data demonstrating efficient transduction of these primary cell lines. Dr. Smith recommended approval of the protocol. Dr. Smith asked the Principal Investigator to comment on the other ongoing melanoma study employing the same retroviral vector (Dr. Seigler's Protocol #9306-043).

Review--Dr. Chase (presented by Dr. Smith)

Dr. Chase's written comments stated that this protocol is similar to other protocols previously approved by the RAC, and the vector and packaging cell line are identical to those approved for Dr. Seigler's study (Protocol #9306-043). Therefore, there are no

significant safety issues of concern. Statistical analysis of this study could be improved if the number of treatment groups is reduced and the number of subjects per group is increased. The study should be limited to autologous cells with 3 dose-escalation groups and 6 patients per dose. The investigators responded that the allogeneic group is necessary because autologous cells may be difficult to obtain from all patients and this disease is very rare. The investigators proposed an alternative design involving 2 dose escalation groups: 3 patients in the low dose group and 6 patients in the high dose group. The Informed Consent document does not stipulate that the sponsoring institution will provide compensation for non-negligent injuries arising from participation in the protocol. The assent form language is not understandable to children. Dr. Chase recommended that the term "vaccine" should be deleted from the Informed Consent document since this protocol is a Phase I study.

Review--Ms. Meyers (presented by Dr. Smith)

Ms. Meyers' written comments raised several concerns regarding the Informed Consent document. She reiterated Dr. Chase's comments about the children's assent form, the use of the term "vaccine," and language relating to the provision of medical care if individuals are injured during the course of their participation in the protocol. An explanation of long-term follow-up was not included. The investigators' written comments noted that the Informed Consent document language is in accordance with the IRB's policy. Since there is the possibility of benefit to subjects, the institution should not have the responsibility of funding treatment for non-negligent complications. Ms. Meyers' written response to the Principal Investigators noted that a Phase I study is not designed to provide benefit to patients.

Other Comments

Dr. Zallen asked about the time frame in which patients receiving allogeneic cells would be informed about their eligibility to participate in the study based on HLA typing. Has HLA typing been completed for all of the cell lines in the cell bank? In regard to the Informed Consent document, the investigators should explain the statement that subjects will be responsible for all costs except for the experimental "vaccine" itself. Dr. Smith added that there is the potential for long-term survival of participants in this study; therefore, long-term care is a pertinent issue.

In response to the RAC's discussion regarding the issue of compensation for research related injuries, Dr. Wivel (Executive Secretary) referred to a letter dated January 28, 1994, from Mr. Robert B. Lanman, NIH Legal Advisor. Mr. Lanman stated that 45 Code of Federal Regulations Section 46.116(a)(6) governs the use of human subjects in research conducted or supported by the Department of Health and Human Services (DHHS). These regulations require that when research involving more than minimal risk is proposed, the subjects of the research must be provided with an explanation as to whether any compensation or any medical treatments are available if injury occurs, what such compensation consists of, and where further information may be obtained. Thus, the RAC's recommendations relating to research-related medical compensation is

contrary to the regulations. The institution is required to disclose such policy to any potential participant.

Drs. Parkman and Straus agreed that the RAC should not dwell on this issue during the deliberation of each protocol, citing unfairness to investigators. Dr. Straus stated that although the RAC has the persuasive power to change policy, the committee does not have the legal authority to demand local IRBs to incorporate such changes in their Informed Consent documents.

Investigators' Responses--Drs. Seeger and Rosenblatt

In response to Dr. Zallen's questions about HLA typing, Dr. Seeger responded that 140 cryopreserved tumor cell lines are currently in the process of being HLA typed. From this cell bank, a panel of common HLA types will be identified, e.g., HLA-A2. Approximately 40% of all individuals are HLA-A2 positive. An HLA matched cell line is anticipated for most allogeneic patients. The protocol and the Informed Consent document will be revised to reflect a modified eligibility criterion such that subjects with no available autologous tumor cells will receive a single HLA matched allogeneic cell line. If an HLA matched cell line is unavailable, the subject will be ineligible to participate in the study.

In response to Dr. Zallen's concerns about patient responsibility for research-related costs, Dr. Rosenblatt stated that all of the costs for research will be the responsibility of the institution. Dr. Rosenblatt agreed to revise the assent form in language that is more understandable to children; however, the average age of patients entered on this protocol will be 3 to 4 years old, which is below the age at which a subject can give assent. Most children are diagnosed with neuroblastoma before 7 years of age.

Dr. Walters asked how a determination will be made regarding the assignment of patients to a particular institution. He said that the language regarding compensation for research related injury in the Informed Consent documents differs between the University of California at Los Angeles and Children's Hospital of Los Angeles. Dr. Seeger responded that most patients will choose the institution where they will receive their care but others will be referred from outside institutions.

Committee Motion

The RAC approved a motion made by Dr. Smith and seconded by Dr. Post to accept the protocol submitted by Dr. Joseph Rosenblatt of the University of California, Los Angeles, California, and Robert Seeger of the Children's Hospital, Los Angeles, California, by a vote of 11 in favor, 1 opposed, and 1 abstention. RAC approval is contingent on the review and approval by the primary RAC reviewers of: (1) a revised protocol that includes an indication of when HLA typing will be performed on subjects considering participation in the allogeneic study, (2) modification of the eligibility criteria to exclude subjects that do not demonstrate a match at any HLA locus, (3) a revised Informed Consent document that includes a statement notifying subjects that they will

not continue on this study if an HLA match at any locus is not identified, and (4) a revised patient assent form written in language that is understandable to children.

Dr. Walters noted that Dr. Parkman abstained from voting on this protocol since he is employed by the same institution as Dr. Seeger, the Children's Hospital, Los Angeles, California.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I/II PILOT STUDY OF THE SAFETY OF THE ADOPTIVE TRANSFER OF SYNGENEIC GENE-MODIFIED CYTOTOXIC T-LYMPHOCYTES IN HIV-INFECTED IDENTICAL TWINS*/DR. WALKER

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the protocol submitted by Dr. Robert E. Walker of the NIH, Bethesda, Maryland. Dr. Post said that this protocol is extremely innovative because of the novel function of the transferred gene; T cells will be activated when HIV antigens are encountered. Specifically, in response to the HIV envelope (*env*) protein. HIV infection progressively destroys the human immune system and ultimately results in acquired immunodeficiency syndrome (AIDS). CD8(+) T cells kill virus infected cells. AIDS may result from a break-down of this immune surveillance system. CD8(+) T cells will be obtained from an uninfected identical twin of an HIV-infected patient and transduced with the vector, ζr*kat*4SVGF3e-, which contains a hybrid gene that activates the signal transduction system for T cell activation. This hybrid gene includes two components: (1) the extracellular domain of human CD4 (the receptor for the HIV envelope protein), and (2) the intracellular domain of the zeta (ζ) chain of the T cell receptor. The transduced T cells will be activated in the presence of HIV envelope proteins without major histocompatibility complex restriction. The genetically modified cells will be purified and expanded to large numbers *in vitro* prior to infusion into the HIV-infected twin. The study is divided into two phases. The first phase involves a single administration of transduced T cells to determine a maximum tolerated safe dose. The second phase involves ≤ 6 infusions of this maximum tolerated dose. Subjects will be evaluated for the safety and tolerance of adoptive immunotherapy with the transduced CD8(+) T cells, including monitoring of their immune status, viral burden, clinical symptoms, organ function, and persistence of circulating marked cells. This study will provide baseline information for future studies.

Dr. Post explained that the investigators have adequately responded to the following questions raised in his primary written review: (1) Is there preclinical data that supports this protocol? The investigators subsequently provided a manuscript that describes the preclinical studies. (2) Is there additional information available about this new vector? The investigators responded that the vector, ζr*kat*4SVGF3e-, was developed to allow high level expression of the transgene following transduction into human T cells. This vector is similar to the LXSN vector previously approved by the RAC; however, a

modification has been introduced at the splice acceptor site which permits expression of the transgene similar to the *env* gene mRNA of the Moloney murine leukemia virus. The investigators have demonstrated high level expression of the hybrid receptor gene in T cells. (3) Will the "ping-pong" method of cocultivation of ecotropic and amphotropic producer clones increase the probability of generating RCR? The investigators have supplied information regarding thorough RCR safety testing. (4) What will happen when large numbers of transduced T cells activated by viral proteins are infused into these HIV(+) patients? The investigators have performed a murine experiment to evaluate this safety issue. A murine tumor cell line was engineered to express the HIV *env* gene, and transplanted in a nude mouse. The mouse was then infused with T cells transduced with the hybrid receptor gene. No serious toxicity was observed in the murine model. Dr. Post cautioned that the solid tumor model is very different from the human situation in which the HIV-infected cells are dispersed throughout the body. This safety issue is significant since the proposed initial cell dose is very high. (5) Will the transduced T cells be susceptible to HIV infection, therefore, expanding the number of HIV-infected cells in the body? The investigators submitted data demonstrating that the transduced cells are at least 1,000-fold less susceptible to viral infection compared to control CD4(+) T cells. Dr. Post suggested that additional data using other clinical HIV isolates is preferable. (6) Will the CD4/ζ hybrid protein become immunogenic? The investigators responded that no immunological responses have been observed in HIV(+) patients who received large quantities of soluble CD4 proteins. Dr. Post recommended that patients should be monitored for anti-CD4 antibodies.

Review--Dr. Carmen

Dr. Carmen commented that the description of the proposed vector and its derivation are not understandable to laypersons because of the numerous undefined acronyms. He recommended specific changes to the Informed Consent document that would more clearly explain the vector and gene insert. As given, the description conveys little useful information to the research subject.

Review--Dr. Brinckerhoff (presented by Dr. Post)

Dr. Brinckerhoff's written comments raised several concerns. The proposed cell doses, 1 x 10^8 and 1 x 10^{10} cells, represent an extremely large number of cells that could result in potential toxicity. How were the initial cell doses determined? The investigators' written response states that the proposed doses are within the range used for other adoptive immunotherapy protocols, e.g., Dr. Rosenberg's (Protocol #9007-003) and Dr. Greenberg's (Protocol #9102-017). Will the hybrid receptor become antigenic? The 7-day experiment demonstrating the lack of transduced T cell infectivity by HIV is inadequate due to the insidious nature of HIV infection, the low level of T cell replication, and a relatively low multiplicity of infection in HIV patients. Is data available from the ongoing 14-day experiment? Efficacy will be difficult to demonstrate since the nature of HIV infection in insidious and variable. The investigators' written response states that the primary endpoint of this study is safety, not efficacy.

Other Comments

Dr. Motulsky asked for additional information regarding the 65 discordant identical twins identified as eligible for this study. This patient population is a valuable resource for other genetic studies. Dr. Parkman inquired about the types and levels of cytokines that are released when the transduced T cells are activated by HIV. Will these cytokines cause adverse effects, e.g., edema, in anatomically defined areas such as the brain? If such a side effect occurs, is therapy available that would alleviate such effects? Is there data demonstrating that steroids cause apoptosis of these clones?

Dr. Zallen inquired whether participation in this study will preclude the 65 discordant twins from participation in future protocols. She recommended that the Informed Consent document be revised to include a statement about long-term follow-up, even if subjects who have received transduced cells terminate their participation in the protocol prematurely. Dr. Hirano asked whether the Phase II portion of this study implies that efficacy is an endpoint.

Dr. Walters called on Ms. Gracia Buffleben of Breast Cancer Action and ACT UP/Golden Gate, to read the written statement submitted by Mr. G'dali Braverman, ACT UP/Golden Gate. Mr. Braverman applauded Cell Genesys (sponsor) for seeking comments on this study from the HIV(+) community. Mr. Braverman's comments focused on scientific issues of the protocol as well as the Informed Consent document. Mr. Braverman suggested the following: (1) potential participants should be informed that participation in this study may exclude their eligibility for other studies, (2) subjects in the control group should be allowed to receive the same treatment as the experimental group once the safety issues have been established, (3) the transduced T cell infectivity experiments should be expanded to include other HIV clinical isolates, (4) lymph node biopsy should be performed, (5) patient eligibility should include females, (6) is the viability of transduced cells affected by freezing and thawing? and (7) patient eligibility should be reduced from 18 to 13 years of age and localized treatment of Kaposi sarcoma should be permitted.

Investigators' Responses--Drs. Walker, Lane, Zsebo, and Roberts

In response to Dr. Carmen's comments about the description of the vector, Dr. Walker agreed to revise these sections of the protocol and the three Informed Consent documents in language that is understandable to laypersons. In response to Dr. Motulsky's question regarding discordant twins, Dr. Walker answered that approximately 10% of the 146 (not 65 as stated earlier) pairs of discordant identical twins enlisted over the last 10 years are female.

Dr. Walker explained that this Phase I/II study is divided into two treatment periods. The first period involves a single infusion of gene modified cells in the experimental group and nonmodified cells in the comparative group. After characterization of toxicities, the second treatment period will involve repeated infusions every 8 weeks for one year. Toxicity, immunological activity, and additional information with regard to

activity of the transduced cells will be obtained. Dr. Hirano questioned whether the study design will allow acquisition of statistically significant efficacy data; and if the primary objective is toxicity assessment, why are so many patients needed. Dr. Walker said that according to the suggestion by his consulting statistician, some statistically significant information regarding efficacy may be obtained by increasing the number of patients from 24 to 40 during the second treatment period. There are many identical twins interested in participating in this study; therefore, recruitment is not a serious problem.

Dr. Smith asked about the number of twins who have developed AIDS. Dr. Walker answered that approximately one-third of these subjects have CD4 counts below 200, one-third between 200-500, and one-third above 500.

Dr. Walker responded to the suggestions outlined in Mr. Braverman's written comments. A statement will be included in the Informed Consent document that advises patients that their participation in this protocol may exclude them from other vaccine immunotherapy or gene therapy studies. With regard to the willingness of subjects to participate in the control group of this study, Dr. Walker explained that once the safety issues have been resolved, protocols will be developed to examine efficacy. Patients in the control arm will receive untransduced CD8(+) cells. A companion protocol is in progress that specifically addresses the issue of lymph node biopsy; therefore, subjects will not be excluded from the study if they refuse such biopsies. Tonsil biopsy will be considered for the present study. Dr. Walker did not agree to lower the age of patients from 18 to 13 since this protocol is not a pediatric study. Regarding the question posed by Dr. Parkman about the management of possible encephalopathy, Dr. Walker responded that an immediate plan has not yet been established for such possible side effects; however, standard medical practices would be implemented.

Dr. Zsebo of Cell Genesys, Inc., Foster City, California, responded to the question on the animal model experiment. Dr. Zsebo said that this experiment was designed to obtain information about efficacy and safety. Two types of experiments have been performed. Tumors were established in nude mice with tumor cells transduced to express the HIV envelope proteins. These tumors were challenged by infusion of mouse T cells transduced with the hybrid CD4 receptor gene. In another experiment, tumor cells and transduced T cells were mixed prior to implantation in nude mice. Notwithstanding the interaction of these two types of cells, no overt inflammatory response was observed. Dr. Zsebo speculated that a probable reason for the negative result might be that not all tumor cells implanted in nude mice were transduced and expressed HIV envelope proteins, rendering them susceptible to cell killing by T cells transduced with the hybrid receptor. Attempts will be made to purify the tumor cells expressing the HIV envelope proteins before implantation in nude mice in order to repeat these experiments. Dr. Post asked whether there is any evidence to indicate that the transduced T cells, which are present in blood circulation, are activated by the HIV proteins expressed on cells in the tumor mass. Dr. Zsebo conceded that this result is a potential shortcoming of the present model. Some other primate and severe combined immunodeficiency mouse models will be considered, but there is no satisfactory animal

model for the present protocol.

Considering the lack of a reliable animal model to assess safety, Dr. Post cautioned that the investigators should proceed with a conservative approach towards this study, i.e., starting at a cell dose of 10^6 instead of 10^8 cells with a small number of patients to assess toxicity. Dr. Walker explained that the proposed cell dose of 10^8 is derived from other studies such as Dr. Greenberg's (Protocol #9202-017). Dr. Post said that in that study, a consideration has been taken that there is potential difference in toxicity in cytomegalovirus (CMV) vs. HIV infection, and a suicide gene has been incorporated in the study.

Dr. Clifford Lane of NIH, Bethesda, Maryland, responded to Dr. Post's comments that there is a significant difference in CMV and HIV infections but it is technically difficult to incorporate a suicide gene in the present study. Responding to Dr. Parkman's question on side effects, Dr. Lane acknowledged that cortisteroids or other immunosuppressive regimens will be considered as first-line intervention in the event of unforseen side effects. In response to the RAC's concern about committing the valuable discordant twin resource to this study, he explained that a statistically significant sample size is important in order to obtain definitive information for the preliminary assessment of efficacy. This initial information will be used to design future studies that directly address efficacy. Dr. Zallen inquired whether there will be enough eligible patients left for future studies after enrolling the large number of patients in this Phase I/II study. Dr. Lane answered that future studies will be designed using autologous cells obtained from HIV-infected individuals; therefore, identical twins will be unnecessary. Dr. Smith suggested that perhaps the present trial should be limited to HIV-infected twins with CD4 counts below 200. Dr. Lane said that it is important to assess any immune based therapy with a spectrum of patients since different outcomes may be observed in patients with different immune status. Dr. Lane agreed to decrease the starting dose from 1×10^8 to 1×10^7 cells in response to safety concerns. Dr. Lane described another preliminary animal experiment designed to assess safety. Human peripheral blood lymphocytes were transplanted into severe combined immunodeficiency mice. Human CD4 counts were measured, and no severe destruction of the immune system was observed following infusion of the transduced T cells.

Dr. Margot Roberts of Cell Genesys, Inc., Foster City, California, explained that HIV infectivity of transduced T cell experiments are in progress using a panel of primary HIV isolates and laboratory HIV strains. In addition, other experiments are being performed to assess the antiviral activity of the transduced T cells. Antiviral activity of T cells expressing the CD4/ζ hybrid receptor has been observed toward cells infected with the strain IIIB of HIV-1. Dr. Parkman inquired about the cytokine profile of the activated T cells. Dr. Roberts explained that the profile of CD4/ζ hybrid receptor activation is similar to activation by the normal T cell receptor, e.g., low levels of gamma-interferon (IFN), β-IFN, granulocyte colony stimulating factor (G-CSF), tumor necrosis factor (TNF)-α, TNF-β, and IL-4. Dr. Parkman noted that toxicity is often associated with certain cytokines, e.g., TNF. Dr. Straus asked whether all or only a small fraction of the transduced T cells when infused into patients will be responsive to HIV antigen

stimulation. Dr. Roberts said that the cytolytic activity is specific; only cells expressing the HIV envelope are killed by the transduced T cells. Dr. Straus expressed his concern about the possible systematic effects of cytokines that may be released when large numbers of transduced T cells activated by HIV in the blood circulation. The amount of cytokine release may be comparable, whether it was triggered through the T cell receptor or the CD4-ζ chimeric receptor.

Dr. Roberts alluded to studies demonstrating that only those cells expressing HIV envelope antigens were killed by activated T cells. The addition of "innocent" cells indicated that they were not targets for lysis. Thus, it was concluded that it was unlikely that there would be problems associated with nonspecific activity. The use of dose escalation studies in patients will allow for careful monitoring of responses.

Dr. Parkman noted the importance of quantitating cytokine release in order to determine a valid starting dose. Dr. Post suggested that the RAC approve a preliminary study on a small group of patients at a starting cell dose of 1×10^7 transduced T cells in order to assess toxicity. If 1×10^7 cells is determined to be a safe dose, the investigators could proceed with the dose-escalation study as originally proposed. Dr. Parkman suggested that the RAC should require submission of quantitative cytokine release data as a stipulation for approval in order to properly assess an adequate cell starting dose. Dr. Parkman suggested quantitating the cytokines released by 1×10^7 activated cells. Dr. Straus suggested that patients should be monitored for circulating plasma cytokine levels following cell infusion.

Committee Motion

The RAC approved a motion made by Dr. Post and seconded by Dr. Smith to accept the protocol submitted by Dr. Robert Walker of the NIH, Bethesda, Maryland, by a vote of 12 in favor, 0 opposed, and 1 abstention. RAC approval of the protocol is contingent on review and approval of the following by the primary reviewers: (1) a revised experimental design that includes a treatment group that will receive a single administration of 1×10^7 HIV-specific CD8(+) cells (the number of subjects to be determined by the investigator) in addition to the 40 patients who will receive multiple doses of between 1×10^8 and 1×10^{10} cells; (2) inclusion of a statement in the protocol that addresses possible treatments available in the event of unforseen toxicity, e.g., encephalitis; (3) data demonstrating that expression of the universal receptor in a panel of primary clinical isolates obtained from HIV(+) individuals does not increase the susceptibility of these cells to HIV infection and data derived from ongoing 14-day experiments involving cell proliferation versus viral replication; (4) quantitative data derived from *in vitro* experiments demonstrating the amount of cytokine(s) produced/1×10^7 cells/24 hours; (5) inclusion of a statement in the protocol which describes the addition of anti-CD4 antibody monitoring; and (6) a revised Informed Consent document incorporating modifications submitted by Dr. Carmen and "in the spirit of" Mr. G'dali Braverman's comments.

Dr. Walters noted that Dr. Straus abstained from voting on this protocol since he is

employed by the same institution as Dr. Walker, NIH.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *EXPRESSION OF AN EXOGENOUSLY ADMINISTERED HUMAN ALPHA-1 ANTITRYPSIN GENE IN THE RESPIRATORY TRACT OF HUMANS*/DR. BRIGHAM

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Kenneth L. Brigham of Vanderbilt University, Nashville, Tennessee. Dr. Parkman stated that the alpha-1-antitrypsin (AAT) protein is produced by normal lung cells to neutralize the damaging proteolytic enzymes. Two clinical settings are proposed for this study: (1) patients who have inherited a congenital defect which prohibits AAT production, and (2) patients with adult respiratory distress syndrome. Patients who inherit a defective AAT gene develop emphysema at an early age. Patients with adult respiration distress syndrome release large amounts of digestive enzymes that can overwhelm the protective effect of AAT as a result of infection, trauma, etc. The basis of this proposal is that lung cells may be protected from the damaging effects of excessive levels of digestive enzymes, whether from an inherited deficiency or as a result of acute damage, by genetically modifying these cells to produce increased levels of AAT. The investigators propose to transfer the AAT gene into the nasal and lower airway cells using a plasmid DNA/liposome delivery system. The non-viral plasmid DNA construct, pCMV4-AAT, consists of promoters, enhancers, and RNA processing sites for the expression of human AAT. Individuals with congenital AAT deficiency will be monitored for local AAT expression following administration of pCMV4-AAT to the nasal mucosa. Patients scheduled for partial or total lung resection for cancer within 2 or 3 days will be monitored for the presence and expression of pCMB4-AAT in resected lung tissue.

Dr. Parkman explained that the gene delivery approach is very similar to the methods used for the cystic fibrosis protocols that were previously approved by the RAC. However, the investigators have submitted insufficient data demonstrating transduction efficiency and adequate expression of the AAT gene. Transduction efficiency was successfully demonstrated in the cystic fibrosis protocols by visualization of the blue color which resulted from the transduction of the reporter gene, β-galactosidase, on the surface of the trachea and lung. Although the investigators have provided immunohistochemical data on cross sections of lung tissue for the current proposal, it is difficult to estimate the percentage of epithelial cells that express the AAT gene. The investigators' written response to Dr. Parkman's concerns state that in contrast to the cystic fibrosis transmembrane conductance regulator protein, AAT is a secreted protein; therefore, the efficacy of gene transfer will depend more on the localization and quantity of protein expressed than on the fraction of cells transfected. Dr. Parkman reiterated his predilection regarding demonstration of efficient transduction and its importance for this *in vivo* gene transfer experiment. A preferable method would be to stain for the expression of the transgene in the mucosal surface of a resected bronchus to estimate the

percentage of transduced epithelial cells. As compared with previously approved cystic fibrosis protocols, the preclinical animal studies of the present protocol are inadequate. Dr. Parkman recommended that the investigators should be required to provide additional data demonstrating the frequency of transduction in both bronchial and nasal mucosal cells in a preclinical animal model.

Review--Dr. Miller (presented by Dr. Parkman)

Dr. Miller's written comments stated that this protocol employs a non-viral plasmid DNA vector which does not pose any significant risk from a recombinant DNA aspect. The Biosafety Level (BL) 2 containment, which was recommended by the Institutional Biosafety Committee for the animal studies, is overly stringent and should be reduced to BL1 containment. The key issue with regard to clinical application is the level of AAT production. Normal individuals express very high endogenous levels of AAT and AAT deficient individuals express low levels of AAT; therefore, expression of the AAT transgene may not be distinguishable from endogenous levels. While the investigators can conduct *in situ* vector RNA analysis, pathology studies, etc., quantitative data about vector-encoded AAT expression may be elusive. Dr. Miller suggested that the nasal mucosa experiment should be performed in large animals rather than in humans.

Review--Dr. Zallen

Dr. Zallen raised several concerns about the experimental design of this study in terms of risks and benefits, the informed consent process, and Informed Consent document. In her written review, Dr. Zallen questioned why tissues will be examined 72 hours after vector administration in the proposed human study, but the preclinical animal studies were conducted at 24 hours. Why were the animal experiments not extended to 72 hours to obtain comparable data? In their written response, the investigators stated that the protocol would be modified for the human experiment to include tissue examination between 24 and 48 hours following vector administration. How many patients will be entered onto the study? In their written response, the investigators stated that 5 patients will be entered on the bronchial instillation protocol and 6 patients will be entered on the nasal instillation protocol. Dr. Zallen expressed concern about possible conflict of interest as a result of investigators obtaining informed consent from their own patients. Under such circumstances, patients could feel obligated to participate in this study. A third party should be involved in the informed consent process. The investigators should elaborate on their comments regarding the lack of necessity for long-term follow-up. She noted Ms. Meyers' written comments about the failure to include information about alternative enzyme replacement (prolastin therapy) in the Informed Consent document.

Other Comments

Ms. Grossman agreed with Drs. Parkman and Millers' recommendations about the necessity for additional preclinical data derived from an appropriate animal model. Dr. DeLeon asked how expression of the transgene will be distinguished from endogenous AAT expression. Dr. Parkman explained that the investigators will conduct *in situ* RNA

analysis to distinguish endogenous versus transgene expression.

Investigator Response--Dr. Brigham

Dr. Brigham responded to the questions concerning transduction efficiency and *in vivo* expression of the transgene in preclinical studies. He presented data demonstrating histochemical staining of the entire airway epithelium of rabbits with an AAT antibody following aerosol delivery of the DNA/liposome complex. Dr. Parkman commented that although transgene expression is demonstrated in cross sections obtained from airway tissue, a three-dimensional analysis is preferable. Dr. Brigham expressed his reservations about using the β-galactosidase reporter gene to demonstrate transgene expression in the surface of bronchial epithelium, citing the possibility of obtaining false positive results. Serial cross sections will reveal expression in a three-dimensional sense. Dr. Brigham noted that *in vitro* and *in vivo* experiments have been published indicating that 10 to 15% of cells express the AAT protein. Dr. Parkman noted that the RAC has not had the opportunity to review this data and recommended that such data should be submitted as a stipulation for approval. Dr. Brigham agreed to submit the requested data with the reporter gene. Dr. Brigham presented *in vitro* data demonstrating protection against protease digestion of bronchial epithelial cells by the AAT transgene. Detection of transgene expression in normal individuals is complicated by the fact that normal lungs have high levels of endogenous AAT activity. Since the lungs are not the normal source of AAT synthesis (the protein is made in the liver and transported to the lungs), it is possible to demonstrate *de novo* synthesis of AAT by the transgene in organ cultures of the lung. The DNA/liposome complex will be instilled via bronchoscope to a distal wedge of the lung. Following resection, the tissue will be examined by serial cross sections to detect *in vivo* transgene expression. Histological toxicity data will be obtained regarding inflammatory responses.

Dr. Parkman reviewed the published protein expression data described previously by Dr. Brigham. Following bronchial administration, approximately one-third of the rabbit epithelial cells express the human AAT protein as demonstrated by fluorescence labelling. The transgene was detected in the liver of rabbits 90 minutes following aerosol administration of DNA/liposome complex to the lungs. Such data raises questions about the possibility of germ-line integration and the necessity of long-term follow-up since some of the AAT deficient patients will have long life spans.

Dr. Brigham said that systemic distribution of vector DNA is a legitimate concern; however, the rabbit experiments involved aerosol delivery to the entire lungs and not the localized delivery proposed for the human protocol. Vector DNA was not detected in either the ovaries or testes of animals via intravenous delivery of DNA/liposome complexes; therefore, germ-line transmission is not a concern. Dr. Parkman suggested inclusion of a statement in the Informed Consent document about the possibility of low level systemic absorption. Dr. Brigham agreed to include such a statement.

In response to the issues raised by Dr. Zallen, Dr. Brigham stated that: (1) the experimental period for the human study will be reduced from 72 to 48 hours, (2) the

investigators will not obtain informed consent from their own patients, and (3) statements will be included in the Informed Consent document regarding long-term patient follow-up and a request for autopsy. Dr. Parkman explained that due to the experimental nature of gene transfer at present, these requirements are appropriate. Responding to Ms. Meyers' concern about alternative prolastin therapy, Dr. Brigham explained that the majority of patients that enter the study will be receive enzyme replacement therapy. Prolastin will be discontinued only for a period of 1 month during the gene transfer experiment. A 1 month interruption in prolastin therapy will not significantly affect the course of the patients' disease. Dr. Brigham agreed to amend the statement in the Informed Consent document regarding provision of medical care in the event of research-related injury.

Dr. Brigham expressed his opinion regarding the adequacy of the preclinical studies and their pertinence to the human study. He emphasized that extensive expression of the transgene was demonstrated using the same vector proposed for the human study. This AAT protocol differs from the cystic fibrosis studies in several important aspects, i.e., AAT is a secreted protein that functions in small airways and peripheral alveoli of the lungs; therefore, the animal experiments were designed to demonstrate transgene expression in these areas rather than in trachea and large bronchi as was demonstrated for the cystic fibrosis protocols. The liposome delivery method is not novel and has been approved by the RAC for several other protocols.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Secundy to approve the protocol contingent on the review and approval of the following by the primary reviewers: (1) transduction efficiency data demonstrating a rate of 10-15% transduction *in situ*, and (2) a revised Informed Consent document that includes statements describing the possibility of systemic absorption of vector DNA, the necessity for long term follow-up, and a request for autopsy. The motion for approval passed by a vote of 12 in favor, 0 opposed, and 1 abstention.

Dr. Post abstained from voting due to a conflict of interest (collaborates with Dr. Leaf Huang, a co-investigator on this study).

VIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *USE OF A RETROVIRAL VECTOR TO STUDY THE TRAFFICKING PATTERNS OF PURIFIED OVARIAN TUMOR INFILTRATING LYMPHOCYTES (TIL) USED IN INTRAPERITONEAL ADOPTIVE IMMUNOTHERAPY OF OVARIAN CANCER PATIENTS - A PILOT STUDY*/DR. FREEDMAN

Review--Dr. Brinckerhoff (presented by Dr. Dronamraju)

Dr. Walters called on Dr. Dronamraju to summarize Dr. Brinckerhoff's written primary review of the protocol submitted by Dr. Ralph Freedman of MD Anderson Cancer

Center, Houston, Texas. This protocol is a resubmission of the proposal that was deferred by the RAC at its June 7-8, 1993, meeting. The RAC deferred the original proposal based on the following: (1) inadequate data demonstrating efficient TIL transduction; (2) insufficient data demonstrating selectivity, i.e., specific trafficking of TIL to tumor sites; (3) incomplete statistical analysis; (4) the Informed Consent document must be revised in simplified language; and (5) concerns about patient responsibility for research-related costs must be addressed. The goal of this protocol is to develop TILs that can be adoptively transferred for the treatment of ovarian carcinoma.

Following intraperitoneal administration of neo^R marked TIL, ovarian cancer patients will be monitored for specific migration of TIL to the site of the tumor. The peritoneal fluid and peripheral blood of these patients will be monitored for neo^R and the number of CD8(+) cells will be quantitated at 24 hours, 7 days, and 18 days. The investigators are capable of detecting 1 in 100,000 cells by polymerase chain reaction (PCR) analysis. The preclinical data demonstrated that ovarian TIL, which were expanded in low dose interleukin-2 (IL-2), were CD3(+) and CD8(+) and exhibited preferential killing of autologous ovarian tumor cells. A preliminary study involving TIL and IL-2 administration to patients with advanced refractory ovarian carcinoma demonstrated an increase in radioactive uptake in patients' liver metastases. In this resubmission, the investigators have provided marginal data demonstrating their ability to detect 1 in 100,000 neo^R marked TIL. In Dr. Brinckerhoff's written comments, she states that the protocol is poorly written and the rationale for the study is poorly stated. Why has the proposed number of patients been reduced from 20 to 10? How will these patients be selected? The investigators' written response to Dr. Brinckerhoff's comments state that they are able to obtain 10 to 12% transduction efficiency; however, supporting data was not submitted. Most of the issues raised by Dr. Brinckerhoff remain outstanding.

Review--Dr. Dronamraju

Dr. Dronamraju raised several issues that must be addressed by the investigators: (1) the statement in the Informed Consent document that explains that the patient cannot be reimbursed for any costs associated with research related injuries, (2) the transduction efficiency must be supported by adequate data, (3) data must be submitted demonstrating that TIL trafficking can be distinguished between tumor and adjacent normal tissues by PCR, and (4) the investigator's statement regarding lack of necessity in determining whether transgene expression should be clarified.

Review--Dr. Secundy

Dr. Secundy commented that the Informed Consent document was not written in language understandable to laypersons. There is no clear description of the experiments that will be performed, and there are several inconsistences between the Informed Consent document and the protocol, e.g., possible side effects.

Dr. Secundy summarized the written comments submitted by Ms. Meyers. The Informed Consent document language should be simplified. Patients should not be required to

cover any costs related to research.

Other Comments

Dr. Parkman explained that the 10 to 12% transduction rate noted by the investigators refers to experiments conducted with cell lines rather than primary cultures; therefore, these results probably do not translate to the clinical setting. The investigators must address whether transduction affects the cytolytic activity of T lymphocytes. Tumor-specific trafficking of neo^R marked T cells should be compared to surrounding normal tissue that has a blood supply comparable to the tumor. The marker gene may persist longer in blood cells than in tumor cells.

Dr. Post stated that the protocol is very confusing and asked the investigators to provide a clear description of all clinical and experimental procedures.

Dr. Zallen agreed with Dr. Secundy's assessment that the Informed Consent document is written in language that is not understandable to laypersons. Dr. Zallen stated that the section explaining that patients will be responsible for some of the research costs is unacceptable. The investigators should provide a detailed description of the informed consent process.

Investigator Response--Dr. Freedman

As a point of clarification, Dr. Freedman stated that two separate protocols and Informed Consent documents were submitted, one for the ongoing TIL protocol and the other for the gene marking study. No patient will be entered onto the gene marking study unless he/she has previously been entered onto the TIL study. The objective of the gene marking study is to determine whether neo^R marked TIL can be detected at the tumor site 3 months following TIL administration. Three months after infusion of TIL cells, samples will be obtained by laparoscopy. A total of 10 patients will receive neo^R marked TIL. Dr. Parkman noted the neo^R gene was not detectable in melanoma patients 3 months post-infusion in Dr. Rosenberg's RAC-approved protocol. Dr. Parkman expressed his concern about the proposed experimental design. The investigators propose a single 90 day time point to detect neo^R marked TIL. Dr. Freedman responded that this 3 month period is dictated by the ongoing TIL protocol because this time point is the optimal time period to examine the therapeutic effects of TIL. Patients should not be required to undergo an additional laparoscopy in order to obtain tissue samples. This study would be very difficult to redesign. Dr. Smith inquired about the number of patients that will be evaluable at 3 months. Dr. Freedman answered that approximately 75% of the patients should be evaluable.

Dr. Parkman expressed his concern that there is no study indicating that the marked TIL cells are detectable 90 days post-infusion. There may be no useful information to be obtained by examining the TIL cells at a single time point in the present study design. Dr. Parkman said that a shorter time point such as 30 days appears to be preferable.

Dr. Parkman said that most of the marked TIL cells will be in the blood circulation; therefore, blood supply of the tissue samples will be critically important. TIL activity should be compared by biopsy of the omentum versus normal peritoneal tissue since the amount of blood supply will vary at different locations.

Dr. Freedman presented data demonstrating the level of sensitivity of the PCR assay. PCR analysis detects 1 in 100,000 neo^R marked cells. Dr. Smith expressed his concern whether 10 patients would yield statistically significant information. Dr. Parkman explained that normal peritoneal tissue obtained by laparoscopy may not represent the best tissue for comparison since the blood supply is significantly different than that of the tumor.

Dr. Chris Platsoucas of Temple University responded to questions about transduction efficiency. The 10 to 12% transduction efficiency data refers to primary TIL cells rather than to cultured cell lines. With regard to transgene expression, Dr. Freedman explained that this protocol is a marking study; therefore, expression is of secondary interest.

Dr. Freedman responded to concerns about patient responsibility for some of the research costs. This statement in question was required by the MD Anderson Cancer Center's IRB and its legal counsel. Dr. Secundy asked whether such a statement is required for all MD Anderson Cancer Center protocols. Dr. Walters noted that this statement was not included in other MD Anderson Cancer Center gene marking protocols previously reviewed by the RAC.

Dr. Post asked about the experimental design of this study. The schema combined treatment flow charts of an ongoing TIL therapy protocol with the present gene marking study. Drs. Post, Smith, Parkman, and Straus asked many questions in order to clarify how patients will be infused with the marked TIL cells and how samples will be obtained and analyzed. Drs. Freedman and Platsoucas attempted to clarify the uncertainty about the treatment schema. Dr. Motulsky recommended that the investigators submit a revised protocol incorporating all of the suggestions referred by the RAC. Dr. Parkman said that the revised protocol should include a scientific rationale that supports tissue sampling 90 days post-infusion. Do the neo^R sequences persist at 90 days?

Dr. Secundy recommended that a revised Informed Consent document should be required that includes a clear rationale as to the purpose and schedule for all clinical procedures and a description of any possible risks and side effects of these procedures.

Committee Motion

A motion was made by Dr. Motulsky and seconded by Ms. Grossman to defer approval of the protocol until the investigators return to the full RAC with the following: (1) a modified protocol that includes a revised treatment schema, and (2) a revised Informed Consent document that describes the clinical procedures to be performed in language that is understandable to laypersons. The motion to defer the protocol passed by a vote of 12 in favor, 1 opposed, and no abstentions.

IX. REPORT FROM THE RAC WORKING GROUP ON VACCINES--AMENDMENTS TO FOOTNOTE 21 OF THE *NIH GUIDELINES* REGARDING THE DEFINITION OF RECOMBINANT DNA VACCINES/DR. POST

Dr. Walters called on Dr. Post, Chair of the RAC Working Group on Vaccines, to provide background information regarding the proposed amendment to Footnote 21 of the *NIH Guidelines*. Dr. Post explained that the current Footnote 21, which defines experiments involving the administration of recombinant DNA to human subjects that are exempt from the *NIH Guidelines*, was adopted in 1986. The original definition of Footnote 21 is outdated due to the scientific advances in recombinant DNA technology. The Working Group on Vaccines (Drs. Post, Parkman, and Straus) was established to formulate a revised definition of Footnote 21. The amended version of Footnote 21 (Version A) reads:

> Version A: "Experiments where the induction or enhancement of an immune response to a vector-encoded immunogen is the major therapeutic goal, and such an immune response has been demonstrated in model systems, are not covered under Section III-A-4 of the Guidelines. Such experiments can occur without RAC review if a Federal regulatory agency has approved the experiments."

The proposed definition emphasizes the immune response to a "vector-encoded immunogen;" therefore, other cellular immunogens would be excluded, e.g., genetically modified cells expressing IL-2 and cells modified to express a histocompatibility antigen. The proposed definition is intended to include immunogens encoded by vectors derived from vaccinia viruses, adenoviruses, and retroviruses.

Drs. Parkman and Straus proposed an alternative definition (Version B) for Footnote 21.

> Version B: "Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in a model system, and the persistence of the vector-encoded immunogen is not expected, are not covered under Section III-A-4 of the *NIH Guidelines*. Such studies can be initiated without RAC review if approved by another Federal regulatory agency."

Dr. Post suggested that the term "persistence" should be removed from Version B since persistence is difficult to define, e.g., adenovirus and retrovirus vectors have been shown to persist to some extent in humans.

Dr. Parkman explained that for the purposes of Footnote 21, "persistence" is defined as whether the construct is intended to be expressed for a significant period of time in order to achieve prolonged stimulation that elicits an immune response. The investigator's intent regarding persistent expression supersedes any residual biochemical persistence. One example of an exempt vaccine covered by Version B would be a retrovirus construct encoding an HIV antigen that is not intended to persist. Although there may be biochemical persistence, the experiment is considered exempt from the

NIH Guidelines. Under Version B, Dr. Richard Haubrich's human gene transfer experiment (Protocol #9312-062) would have been exempt from RAC review. Dr. Haubrich's study involved the intramuscular injection of the retrovirus vector, HIV-IT(V), which encodes HIV-1 IIIB *env*. Version B would require long-term expression vaccines, e.g., certain influenza vaccines to be submitted for full RAC review.

Dr. Straus compared the differences between Versions A and B of Footnote 21: (1) Version B includes the term "human studies," which narrows the definition. (2) Version B includes the term "microbial immunogen," which limits the exemption to microbial immunogens and excludes other gene products. (3) Version A deletes the term "therapeutic," since the majority of vaccines are intended for prophylactic rather than therapeutic purposes. (4) Version B includes the term "persistence." Dr. Straus expressed his concern that the RAC should not exempt experiments in which the vector would persist or encode immunogens other than those of microbial origin. Dr. Post asked if the term "microbial" would encompass viruses. Dr. Straus responded that viruses would be considered "microbial."

Dr. Straus disagreed with Dr. Parkman's interpretation of the term "persistence." "Persistence" would be defined as biochemical persistence of the vector, not the immune response. Herpesviruses and adenoviruses are capable of long-term persistence in the body; therefore, not all constructs involving these viruses should be considered exempt. Dr. Walters asked whether Version B expands the scope of RAC review, e.g., including adenovirus vaccines, that have not been reviewed by the RAC previously. Dr. Straus acknowledged that Version B would exclude adenovirus vaccines; therefore, RAC review would be required. Ms. Grossman expressed her concern about adenovirus vectors. The wild-type virus persists in lymphocytes and different mutations of the virus introduced during construction of adenovirus vectors affect its persistence and immunological activity in the human body. Dr. Parkman agreed that these are reasons that adenovirus vectors should be reviewed by the RAC. Dr. Straus emphasized that advances in technology pose new safety issues, and that he would favor that these issues be resolved by the RAC in the public forum before exempting these vaccine constructs. Dr. Post said that vaccines exempted from the RAC review will still need to be approved by another government agency. Dr. Parkman explained that the majority of the recombinant viral vaccines that exhibit transient expression will be exempt from RAC review, e.g., poxvirus vectors. However, virus vectors that have the potential to persist in the body will not be exempt from RAC review, e.g., retroviruses, adenoviruses, herpesviruses, and papovaviruses. Vaccines involving persistent viruses may be encompassed by the *Accelerated Review* process that is proposed as a separate agenda item for this meeting.

Committee Motion

A motion was made by Dr. Carmen and seconded by Ms. Grossman to approve Version B definition of Footnote 21 of the *NIH Guidelines* (exempt recombinant DNA vaccines). The motion to accept Version B passed by a vote of 13 in favor, 0 opposed, and no abstentions.

Footnote 21 will be amended to read:

> "Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are not covered under Section III-A-4 of the *NIH Guidelines*. Such studies may be initiated without RAC review if approved by another Federal regulatory agency."

X. REPORT FROM THE RAC WORKING GROUP ON ACCELERATED REVIEW--AMENDMENT TO THE *NIH GUIDELINES* AND THE *POINTS TO CONSIDER* REGARDING ACCELERATED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DR. PARKMAN

Minor Actions--Dr. Parkman

Dr. Walters called on Dr. Parkman, Chair of the RAC Working Group on *Accelerated Review*, to summarize the proposed amendments to the *NIH Guidelines* and *Points to Consider*. Dr. Parkman presented a flow diagram that outlined the proposed NIH review process for human gene transfer experiments. The proposed amendments define three categories of review: (1) RAC *Major Actions*, (2) NIH/ORDA *Minor Actions*, and (3) *NIH Director Actions*. Human gene transfer experiments considered as *Major Actions* are described under proposed Section IV-C-1-b-(1) of the *NIH Guidelines*. *Major Actions* require *Federal Register* announcement 15 days prior to the RAC meeting at which the proposal will be reviewed and an opportunity for public comment, full RAC review, and NIH Director approval. Human gene transfer experiments considered as *Minor Actions* are described under proposed Section IV-C-1-b-(2) of the *NIH Guidelines*. NIH/ORDA will determine whether a protocol qualifies as a *Minor Action* in consultation with the RAC Chair and one or more RAC members as necessary. A human gene transfer experiment that qualifies as an *NIH Director Action* is considered under proposed Appendix M-VI, *Single Patient Expedited Review Protocols*. A protocol considered as a *Single Patient Expedited Review Protocol* must be reviewed by extramural experts (may include intramural experts) and approved by the NIH Director. Protocols approved under the *Minor Action* and *Single Patient Expedited Review* categories will be reported by the RAC Chair at the next scheduled RAC meeting. Principal investigators of protocols approved under all review categories will be required to comply with the semi-annual data and adverse effect reporting requirements.

Dr. Parkman said that an additional category has been created for potential future use. These will be experiments that can be initiated simply by registration with NIH/ORDA, and the progress of the study will be tracked by the RAC.

Dr. Straus asked what kinds of experiments will be encompassed under the *Minor Actions* category. Dr. Parkman said that several categories of experiments will be discussed later, such as those involving lethally irradiated tumor cells. Another possible example will be a protocol moving from Phase I to Phase II and III trials. Some protocols will be moved

from *Major* to *Minor Actions*; and in the future, possibly to the registration category. Dr. Straus suggested a minor change to the flow chart describing the review process regarding reporting these actions to the next RAC meeting by the Chair. Dr. Parkman said that the NIH Director will have to make the decision to delegate authority to the RAC to approve *Minor Actions*. Dr. Wivel stated that a *Minor Action* is different from the single patient *Expedited Review* category, which requires approval from the NIH Director. All human experiments involving recombinant DNA will be tracked by the RAC except those vaccine studies that are considered exempt under Footnote 21 of the *NIH Guidelines*. Dr. Smith asked whether the *Minor Actions* will require IBC and IRB approvals prior to submission to ORDA. Dr. Parkman said that prior local approvals will be a condition for submission to ORDA.

Committee Motion--Minor Action

The RAC approved a motion made by Dr. Carmen and seconded by Dr. Straus to accept the amendments to the *NIH Guidelines* and the *Points to Consider* by a vote of 13 in favor, 0 opposed, and no abstentions. The proposed amendments will: (1) establish an *Accelerated Review* process (*Minor Actions*) for certain categories of human gene transfer experiments, (2) allow NIH/ORDA to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with the *NIH Guidelines*, and (3) allow NIH/ORDA to approve those categories of human gene transfer experiments that qualify as *Minor Actions* in consultation with the RAC Chair and one or more RAC members, as necessary. At their discretion, the RAC Chair and/or NIH/ORDA may determine that any proposal shall require review by the full RAC (*Major Action*).

Proposed Categories (Minor Actions)--Dr. Parkman

Dr. Parkman presented a draft document entitled: *Proposed Categories for Minor Actions to the NIH Guidelines Involving Human Subjects*. The proposed categories are considered guidelines for consideration and are not intended as a set of fixed categories. The proposed categories of *Minor Actions* are: (1) vaccines that are not considered exempt under Footnote 21, (2) lethally irradiated tumor cells/no replication-competent virus, (3) additional sites, (4) new Principal Investigator/new site, (5) "umbrella" protocols, (6) modifications not related to gene transfer, and (7) gene marking protocols.

The risk/benefit ratio will be applied to each individual protocol. Dr. Parkman described a hypothetical protocol in which irradiated retinoblastoma cells genetically modified to produce IL-2 will be administered to a child's eye. Although this experiment would be encompassed by the lethally irradiated tumor cell category, the serious clinical setting represents an increased risk/benefit ratio that would require an increased level of review, i.e., *Major Action*. However, the majority of experiments proposed under this category would qualify as *Minor Actions*. Dr. Wivel cited an example of injecting a plasmid DNA vector to a subcutaneous tumor mass vs. administering the vector DNA through a catheter to a lung metastasis. Dr. Parkman said that a protocol such as subcutaneous administration of irradiated cells producing IL-2 to a new tumor type such

as prostate cancer would qualify for *Accelerated Review*.

Dr. Secundy and Ms. Grossman suggested that the working group define the criteria for *Minor Actions* to avoid arbitrary decisions. Dr. Parkman explained that establishing very rigid criteria would exclude a large number of proposals.

The RAC discussed the types of experiments that would be considered under the lethally irradiated tumor cell category. Dr. Parkman said that there are several possible options under this category: (1) a RAC-approved vector with an approved gene insert, (2) a RAC-approved vector with a new gene insert, (3) a modified RAC-approved vector, (4) a new tumor type, and (5) a new route of irradiated tumor cell administration. There should be no replication competent virus.

The RAC considered previously approved proposals initiated at new sites and/or by new Principal Investigators. Dr. Straus and Ms. Grossman expressed their concern that new investigators may not possess adequate qualifications to conduct a duplicate study, and that such a situation may pose increased risk to the patients or the environment. Dr. Motulsky agreed that adequate qualifications is a major issue. Drs. Post, Parkman, and DeLeon assured the RAC that any proposal that presents such concerns can be elevated to the next full level of review, i.e., *Major Actions*.

Dr. Wivel raised a question regarding quality control in umbrella protocols. Would a single Principal Investigator be responsible for all study sites or a different Principal Investigator for each site? Dr. Parkman explained that the RAC-approved *Herpes simplex* virus thymidine kinase/Ganciclovir protocols to treat brain tumors are an example of studies that may qualify for *Accelerated Review*. The vector producing cells are prepared at a central facility and distributed to each site for administration to the patients by neurosurgeons; recombinant DNA expertise is not required in such a situation. Dr. Straus said that local expertise may be required at each site to perform assays such as examining the persistence of vector sequences in patients and assuring that these biological agents are not inadvertently released to the environment. Ms. Grossman agreed that issue is important. Dr. DeLeon said that competence of the Principal Investigator at each site will be a question for review. Dr. Post said that these are issues to be considered when the protocol is submitted for *Accelerated Review* and the RAC does not have to decide at this time.

With regard to protocols involving lethally irradiated tumor cells, Dr. Post said that such studies should be limited to RAC-approved vectors. Dr. Merchant asked whether direct *in vivo* administration of a vector to the tumor site would be considered a *Minor Action*. Dr. Parkman said that if the proposal represents any substantial change over a previously approved study, the experiment would be reviewed as a *Major Action*. Dr. Carmen asked whether the RAC-approved vector/new gene insert category includes any cDNA insert. Dr. Carmen expressed his concern regarding blanket inclusion of any cDNA insert even if the tumor cells are lethally irradiated. Dr. Parkman said that ORDA should review each proposal independently since certain cDNA inserts may pose risk, e.g., insertion of cDNA encoding for a toxin gene. Drs. Post, Straus, Secundy, and Ms. Grossman

deliberated whether these exceptions should be specified or determined on a case-by-case basis when protocols are submitted for review. Dr. Parkman said that it is impossible to list all acceptable cDNA inserts for this category; therefore, the definition should be limited to RAC-approved vector constructs and RAC-approved vector constructs with minor modifications. Dr. Carmen expressed his support for this definition. Dr. Parkman suggested that RAC-approved protocols involving modification of the tumor type should qualify as a *Minor Action*; however, the issue of risk is always a consideration. Dr. Wivel assured the RAC that risk would be considered on a case-by-case basis at the time of submission.

Committee Motion 1 - Proposed Categories

A motion was made by Dr. Carmen and seconded by Dr. Dronamraju to modify the lethally irradiated tumor cell category as follows: lethally irradiated tumor cells/no replication-competent virus, RAC-approved vector constructs with minor modifications/additional tumor cells. The motion to accept this modification passed by a vote of 13 in favor, 0 opposed, and no abstentions.

Committee Motion 2 - Proposed Categories

A motion was made by Dr. Post and seconded by Dr. Straus to approve the following categories of *Minor Actions*: (1) non-exempt vaccines, (2) new site/original Principal Investigator, (3) new site/new Principal Investigator, (4) "umbrella" protocols, and (5) modifications not related to gene transfer. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

Dr. Merchant asked the RAC to consider Phase II and Phase III trials for consideration as *Minor Actions*. Dr. Straus stated that the RAC has considered very few Phase II gene transfer studies; therefore, it is premature to include such experiments as *Minor Actions*. Ms. Grossman and Dr. Parkman agreed with Dr. Straus' statement.

Committee Motion 3 - Proposed Categories

A motion was made by Dr. Smith and seconded by Dr. Post to amend the proposed categories of *Minor Actions* to include gene marking protocols involving RAC-approved vector constructs or RAC-approved vector constructs with minor modifications and/or additional target cells. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

Summary

The *Proposed Categories for Minor Actions to the NIH Guidelines Involving Human Subjects*, as approved by the RAC, reads as follows: (1) Vaccines -- Recombinant DNA vaccines not covered by Footnote 21, (2) Lethally irradiated tumor cells/no replication-competent virus -- RAC-approved vector constructs with minor modifications/additional tumor cells, (3) New site/original Principal Investigator -- RAC-approved protocol

initiated at a new site (the original Principal Investigator is the same for the new site), (4) New site/new Principal Investigator -- RAC-approved protocol initiated at a new site (the Principal Investigator for the new site is different than the Principal Investigator of approved for the original site), (5) "Umbrella" protocols -- RAC-approved protocol initiated at more than one additional site (Principal Investigator may be the same or different than the Principal Investigator approved for the original site), (6) Modification not related to gene transfer -- a modification to the clinical protocol that is not related to the gene transfer portion of the study, and (7) Gene marking protocols -- RAC-approved vector constructs or RAC-approved vector constructs with minor modifications/additional target cells.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING *DELIBERATE TRANSFER OF A CHLORAMPHENICOL RESISTANCE GENE TO AN AVIRULENT STRAIN OF RICKETTSIA PROWAZEKI*/DR. POLICASTRO

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the proposal submitted by Dr. Paul F. Policastro of the NIH, Rocky Mountain Laboratories, Hamilton, Montana. This request was deferred by the RAC at its June 7-8, 1993, meeting until the investigator returned to the full RAC with data demonstrating that the construct is safe and useful, and that there is a selective advantage of using chloramphenicol resistance over other selectable markers. Dr. Post explained that the investigator is requesting permission to introduce the chloramphenicol resistance gene into *Rickettsia prowazeki*. Chloramphenicol is one of the two antibiotics of choice to treat the human infection. This antibiotic resistant gene will be used as a selectable marker to establish a transformation system in eukaryotic host cells. Such studies will be valuable for conducting research on this fatally pathogenic organism. The investigator has responded to the RAC's initial concerns as follows: (1) the study will be confined to *Rickettsia prowazeki* Strain E, (2) a cloning vector is proposed that includes several safety features, and (3) the experiment will be conducted in a BL3 facility. The chloramphenicol resistant strain will be used only to develop the transformation conditions; other selectable markers will be developed to perform subsequent research. Although the proposal is reasonable with regard to the molecular biology aspects, Dr. Post deferred to the opinion of infectious disease experts with regard to safety issues.

Review--Dr. Straus

Dr. Straus explained that Strain E *Rickettsia* is of reduced virulence but is not avirulent. Although Strain E has been used as a human vaccine, the strain is probably capable of reversion to a virulent form after "n" number of *ex vivo* passages. In Dr. Policastro's submission, he states that Strain E has been reported to revert to virulent phenotypes after 13 serial passages in the lungs of albino mice and upon brief passage in pigs under heavy inoculation. Symptoms of human infection with Strain E have been observed following exposure to high titers, i.e., between 1×10^6 and 1×10^7 infectious organisms. The proposed experiment will require manipulating the organism at the levels of 1×10^{11}

infectious organisms or more. The introduction of the chloramphenicol resistance gene will eliminate one of the two antibiotics (chloramphenicol and tetracycline) known to be effective in the treatment of a human infection, louse-borne typhus. Dr. Straus stated that if there are known deletions of the virulence genes, the organism cannot revert to a virulent form, or the organism is crippled in some other way, he would recommend approval of this request; however, considering the significant safety concerns, the RAC should not set a precedent by approving this proposal even for a one-time experiment under BL3 containment.

Other Comments

Dr. Parkman suggested an alternative to disapproving this request. The RAC could recommend approval with the stipulation that any residual bacterial stocks be destroyed once the transformation experiment has been conducted.

Dr. Straus said that there is no absolute scientific reason for inserting the chloramphenicol resistance gene. Although the investigator has stated that chloramphenicol resistance is the selectable marker of choice, other options are available. Dr. Straus said that he would recommend approval of this experiment only in a Biosafety Level 4 containment facility and contingent on the destruction of any residual bacterial stocks once the experiment is performed. The RAC should not set a precedent for this type of experiment.

Dr. Motulsky inquired about the scientific merit of this experiment. Dr. Straus explained that the biology of *Rickettsia* organisms is not well understood. These bacterial pathogens cause serious human disease, e.g., Rocky Mountain Spotted Fever and typhus-like syndromes. This organism has been known to cause devastating illness (typhus) in wartime. Currently, there is no vaccine for typhus. Dr. Straus stated that the objective of such research is extremely valuable; however, a selectable marker other than chloramphenicol resistance would be preferable.

Dr. Post said that in consideration of the serious safety concerns raised by Dr. Straus, this request should be deferred until the investigator proposes an alternative request, e.g., use of a Biosafety Level 4 facility, alternative selectable marker, etc.

Dr. DeLeon asked whether the investigator submitted additional data since the previous review. Dr. Post answered that additional data was not provided, only a more detailed description of the proposed experiment.

Dr. Walters asked whether there is any genetically modified *Rickettsia* that would be less virulent. Dr. Straus said that for many bacteria the virulence genes are not well-defined. He cited a recently published document showing that it is possible to create an experimental environment in which the organism has reduced virulence. Under this condition, experiments using virulent organisms can be performed. It might be worthwhile to attempt to produce avirulence in culture and then transfer the organism to animals where there is the possibility of upregulating certain genes that will restore

virulence.

Dr. Straus said that the investigator has stated that at low multiplicity of infection, this organism (Strain E) causes only mild disease in humans, a condition used for vaccine studies. But in the proposed experiments, the laboratory personnel are expected to be exposed to higher doses of the organism that could cause serious symptoms. Dr. Carmen asked about possible Biosafety Level 4 facilities. Dr. Wivel stated that there are four such facilities in the United States.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. DeLeon to defer the proposal. The proposal was deferred by a vote of 13 in favor, 0 opposed, and no abstentions. The proposal was deferred based on the following: (1) chloramphenicol is one of the two antibiotics of choice for the treatment of *Rickettsia* infection; (2) strain E (proposed for this study) is considered to be of reduced virulence not avirulent; Strain E has been reported to revert to the virulent state after passages *ex vivo*; and (3) data is unavailable demonstrating the probability of reversion to a virulent strain when grown under large-scale conditions, i.e., between 1×10^9 and 1×10^{10} organisms.

The RAC discussed possible scenarios under which this proposal might be eligible for resubmission for RAC review: (1) if the gene encoding for virulence were identified, or (2) the investigator submits a request for use of this organism in a Biosafety Level 4 facility contingent on the destruction of residual stocks upon optimization of transformations assays. The RAC emphasized that resubmission of the proposal will not guarantee RAC approval.

XII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I STUDY OF IMMUNOTHERAPY FOR METASTATIC RENAL CELL CARCINOMA BY DIRECT GENE TRANSFER INTO METASTATIC LESIONS*/DR. VOGELZANG

Review--Dr. Doi

Dr. Walters called on Dr. Doi to present his primary review of the protocol submitted by Dr. Nicholas J. Vogelzang of the University of Chicago, Chicago, Illinois. Dr. Doi explained that the proposed study is very similar to Dr. Gary J. Nabel's (Protocol #9306-045) and Dr. Joseph Rubin's (Protocol #9312-064) previously approved by the RAC. This study will be conducted on 15 HLA-B7 negative patients with metastatic renal cell carcinoma. Patients will receive direct intratumoral injections of a cationic liposome complex containing the plasmid vector, pHLA-B7/β-2 microglobulin. This vector expresses a heterodimeric cell surface protein consisting of HLA-B7 and β-2 microglobulin. Expression of this protein should induce an *in vivo* antitumor immune response. The objectives of this study are to determine a safe and effective dose of the vector, confirm *in vivo* expression, and characterize the immune response. The only difference between this proposal and those of Drs. Nabel and Rubin is the tumor type

(renal cell carcinoma).

Dr. Doi asked the investigators to respond to the following questions: (1) Is novel information expected based on the new tumor type? (2) Would there be an advantage in waiting for the results of Drs. Nabel and Rubin's studies? (3) How many Phase I studies of an identical protocol, but with different tumor types, are required to obtain significant information? He stated that this protocol is an ideal example of an experiment that would qualify for the *Accelerated Review* process, provided that the Informed Consent documents adopted by different institutions are properly reviewed.

Review--Dr. DeLeon

Dr. DeLeon agreed with Dr. Doi's statement regarding the eligibility of this protocol for the *Accelerated Review* process. Dr. DeLeon inquired about the expertise of the investigators at the new site; particularly, the personnel involved in the needle biopsies of the tumor nodules. Dr. DeLeon stated that she was satisfied with responses and additional information provided by the investigators. Dr. DeLeon made several specific suggestions for improving the Informed Consent document: patient follow-up should be life-long and the words, "treatment" and "therapy," should be replaced with "procedure." There are several inconsistencies between the protocol and the Informed Consent document describing the treatment procedures. Dr. DeLeon said that most of the suggestions provided in her written review were accepted by the investigators. Dr. DeLeon recommended approval of the protocol.

Other Comments

Dr. Carmen said that preclinical animal experiments using renal cell carcinoma cells were not provided. Dr. DeLeon noted that extensive animal experiments were submitted to support Dr. Nabel's original protocol. Dr. Parkman explained that based on published data (IL-2 and TIL administration), renal cell carcinoma is the second most responsive tumor type to immunotherapy. Since enhanced antitumor responses have been demonstrated in Dr. Nabel's melanoma protocol, renal cell carcinoma is logically the next tumor to study. Dr. Post said that animal studies are not totally predictive for the human immune response; therefore, animal studies should not be an absolute prerequisite for these human trials.

Ms. Grossman inquired whether Dr. Nabel will have an interactive role with the Principal Investigators at the other sites. Where is the central laboratory located that will perform the immunological assays? How will the results obtained from this multi-center trial be collected and evaluated? How will quality control be assured? Dr. Walters inquired about the most frequent metastatic sites of renal cell carcinoma?

Investigator Responses--Dr. Vogelzang

In response to Dr. DeLeon's question about the qualifications of the investigators, Dr. Vogelzang stated that Dr. Gary Sudakoff will perform the sonographically-guided needle

biopsy. Dr. Sudakoff possesses a great deal of expertise in performing this technique and his biographical sketch has been submitted for review. Responding to Dr. Walters' question about frequent metastatic sites, Dr. Vogelzang said that most renal cell carcinoma metastasis are in visceral organs such as the lung, liver, mediastinal, and retroperitoneal lymph nodes. Such locations are in contrast to the subcutaneous sites of metastases in melanoma patients. In response to Ms. Grossman's question, Dr. Vogelzang stated that Dr. Nabel will not be directly involved in this study. The multi-center trial will be coordinated by Vical, Inc., San Diego, California, and the company has selected a central laboratory to perform the immunological assays. Dr. Parkman asked about the size of the tumor in relation to the dose of DNA to be injected. Dr. Vogelzang said that most metastatic tumors are 2 to 4 cm in diameter. The primary tumors are much larger and necrotic and are not suitable for injection.

Dr. George Gray explained that Dr. Nabel has served on the Scientific Advisory Board of Vical, Inc., and has chaired the Oncology Task Force that decides issues such as the tumor types and institutions that will be involved. Dr. Evan Hersh, Principal Investigator of the Arizona Cancer Center study, will oversee the central laboratory that will perform the immunological assays for all sites. Vical will establish the procedures for testing and evaluate the results from all study sites. Dr. Nabel is conducting an independent protocol that is not considered part of this multi-center study. Dr. Nabel has an investigator sponsored IND, whereas, Vical has an institutional sponsored IND. Dr. Doi asked about the proposed DNA/liposome dose as compared with that of Dr. Nabel's study. Dr. Gray responded that the present dose of 10, 30, and 300 micrograms is bracketed between the highest and the lowest dose of Dr. Nabel's study, i.e., 3 to 500 micrograms.

Dr. Walters stated that the abstracts contain language that implies that there is a therapeutic intent. Such language is not appropriate for a Phase I study. Dr. Vogelzang responded that the abstracts will be modified to reflect this concern.

Committee Motion

A motion was made by Dr. DeLeon and seconded by Dr. Doi to approve the protocol. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

XIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I STUDY OF IMMUNOTHERAPY OF MALIGNANT MELANOMA BY DIRECT GENE TRANSFER*/DRS. HERSH, AKPORIAYE, HARRIS, STOPECK, UNGER, AND WARNEKE

Review--Dr. Doi

Dr. Walters called on Dr. Doi to present his primary review of the protocol submitted by Dr. Evan M. Hersh of the Arizona Cancer Center and Drs. Emmanuel Akporiaye, David Harris, Alison T. Stopeck, Evan C. Unger, and James A. Warneke of the University of

Arizona, Tucson, Arizona. Dr. Doi said that this protocol is similar to other protocols previously approved by the RAC, i.e., Dr. Gary J. Nabel's (Protocol #9306-045), and Dr. Joseph Rubin's (Protocol #9312-064), and Dr. Vogelzang's protocol that was just reviewed and recommended for approval by the RAC. Like Dr. Nabel's protocol, this study involves melanoma patients. However, larger doses of the DNA/liposome complex are proposed for this study. These doses are in the same range as Dr. Rubin's colorectal cancer protocol. This Phase I protocol is a part of the multicenter study sponsored by Vical, Inc., to evaluate the safety and immune responses to direct intratumoral injection of DNA/lipid complexes containing the nonviral plasmid DNA vector, pHLA-B7/β-2 microglobulin. This vector expresses a heterodimeric cell surface histocompatibility antigen which should elicit an *in vivo* antitumor response. Dr. Doi stated that the same comments regarding Dr. Vogelzang's protocol also apply to Dr. Hersh's protocol.

Review--Dr. DeLeon

Dr. DeLeon stated that the term "at two additional sites" should be deleted from the *Points to Consider* since only a single site is proposed for this study, the Arizona Cancer Center. There are several inconsistencies between the protocol and the Informed Consent document with regard to the study design. The protocol states that patients in study Arm 1 will receive a single injection, whereas the Informed Consent document states that multiple injections will be administered to a single nodule (up to 5 times). In the investigators' written response they explained that up to 5 points of injections will be administered into the same tumor mass to maximize contact between the DNA liposome and tumor cells.

Other Comments

Ms. Grossman said that a request for autopsy was inadvertently omitted from the Informed Consent document, and that the number of patients proposed for this study was not clearly stated in the protocol.

Dr. Walters noted a statement in the Informed Consent document regarding compensation for research related injuries. The original document stated, "in the unlikely event of physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment from injuries or side effects directly related to this experimental treatment will be provided at no cost to you." However, the revised document states "Necessary emergency medical care directly related to this treatment will be provided from Evan M. Hersh, M.D." Ms. Grossman inquired about the exact number of patients who will be entered onto the study. Dr. Walters inquired about the reason for this change. Dr. Secundy asked whether a request for autopsy has been included in the revised Informed Consent document.

Investigator Response--Dr. Hersh

Dr. Hersh said that Dr. DeLeon's suggestions regarding "single site" language and a request for autopsy will be incorporated into a revised Informed Consent document.

Responding to Dr. Walters' question about compensation for research injuries, Dr. Hersh said that the revised language was suggested by the IRB of the Arizona Cancer Center. Any medical costs associated with toxic side effects of investigational drugs will be covered by the insurers or third party payers. Regarding the number of patients in the trial, Dr. Hersh said that 15 "evaluable" patients will be entered onto the study.

Dr. Steven Kradjian of Vical responded to Dr. DeLeon's question about the number of study sites. He agreed to submit a revised *Points to Consider* document that specifies a single site, the Arizona Cancer Center. Dr. Hersh said that a request for autopsy will be added to the Informed Consent document and submitted for IRB approval.

Committee Motion

A motion was made by Dr. Doi and seconded by Dr. DeLeon to approve the protocol contingent on review and approval of the following: (1) a revised statement in the Informed Consent document indicating that an autopsy will be requested in the event of death (including IRB approval), and (2) a revised *Points to Consider* document that specifically addresses a single site, the Arizona Cancer Center. The motion to approve the protocol passed by a vote of 13 in favor, 0 opposed, and no abstentions.

There was a follow-up discussion on the issue of autopsy request. Dr. Vogelzang said that the statement was omitted from the Informed Consent document because he considered it improper to require patient autopsy for participation in the trial. Dr. Parkman explained that the RAC does not require an autopsy; however, it is recommended that a statement requesting autopsy should be included in the Informed Consent. Dr. Hersh agreed to coordinate with Dr. Vogelzang to have this statement included in the revised Informed Consent document.

XIV. REPORT ON THE GENE THERAPY ADVISORY COMMITTEE (GTAC) OF THE UNITED KINGDOM/MR. TAYLOR

Mr. Anthony Taylor, Secretariat of GTAC, United Kingdom, provided the RAC with an overview of the activities of this committee which is responsible for review of human gene transfer proposals in the United Kingdom. Mr. Taylor explained the origin of GTAC. GTAC was established following the recommendation of the Clothier Committee in 1991 that endorsed the concept that gene therapy should be considered as a mainstream approach to medical research and should be subjected to all the ethical considerations of medical research. This concept was endorsed at the parliamentary level in the United Kingdom and GTAC was established within the Department of Health to provide oversight of gene therapy research within the United Kingdom. GTAC will report on developments in gene therapy to the Secretary of State for Health. GTAC is chaired by Dr. Judith Lloyd, former Professor of Pediatric Medicine at the University of London. GTAC is composed of 16 members: 8 of whom possess expertise in the areas of medicine and science, and 8 who represent expertise in such areas as clinical psychology, genetic counseling, industry, law, ethics, nursing, and the media. He noted that one member is a leading British Broadcasting Company journalist.

GTAC has approved 6 human gene transfer trials to date; two of these trials are currently in progress, one for the treatment of cystic fibrosis and one for the treatment of adenosine deaminase deficiency (Netherlands collaboration). Studies that have not yet been initiated involve: (1) cytokine-mediated therapy in malignant melanoma, (2) a neuroblastoma marking study (United States collaboration), and (3) a lymphoma vaccine trial. These trials are currently awaiting approval from the Medicines Control Agency (the United Kingdom's equivalent of the Food and Drug Administration). GTAC is currently in the progress of reviewing an additional 4 studies involving cancer, cystic fibrosis, and HIV infection.

GTAC differs from the RAC in that its meetings are not open to the public. However, Principal Investigators are encouraged to publish their protocols in appropriate journals, and GTAC is considering the publication of an annual technical report regarding the activities of the committee. GTAC does not operate under statutory regulations; however, the Department of Health guidelines require that GTAC review all human gene transfer proposals. All biomedical research in the United Kingdom must be reviewed and approved by local research ethics committees.

Dr. Parkman asked how often the GTAC meets and how its review process differs from the RAC review. Mr. Taylor responded that the Secretariat and the Chairman of GTAC screen all human gene transfer studies prior to GTAC review. The committee review is very similar to the RAC review in that one or two primary reviewers are assigned for each proposal. GTAC currently meets 5 times a year; however, this schedule may be modified to accommodate 6 meetings a year. Essentially, a given proposal will be cleared within a 3-month period. Dr. Motulsky inquired whether the gene transfer studies proposed in the United Kingdom differ from those approved by the RAC. Mr. Taylor responded that 3 of the first 6 trials approved by the GTAC utilized non-viable DNA/liposome or plasmid DNA delivery systems. Ms. Grossman asked whether there is any data reporting system to monitor research progress. Mr. Taylor said that the gene therapy trials are still in the very early stage in the United Kingdom and no such system has been in operation yet. Dr. Post asked whether there is any plan to establish a registry to track patients who have received gene therapy. Mr. Taylor responded that the plan to establish a registry is under consideration. Dr. Doi asked about coverage of the medical costs in the United Kingdom regarding gene transfer studies. Mr. Taylor explained that the National Health Service covers all medical costs. Dr. Smith asked whether there is any reimbursement to the National Health Service for trials sponsored by a company. Mr. Taylor responded that there is no system to recoup the costs for company-sponsored research. All the current studies are funded by the government and non-profit charities. Dr. Motulsky asked if Mr. Taylor knew of any additional oversight committees for human gene therapy besides the United States and the United Kingdom, such as other European countries or Japan. Mr. Taylor responded that Japan has recently established a dual mechanism for reviewing gene therapy protocols. In the Netherlands, Denmark, and Germany, protocols are reviewed through existing national advisory committees on genetic modifications.

Dr. Marcel made an unofficial report on gene therapy review in France. There is a

committee under both the Ministry of Research and Ministry of Agriculture that oversees viral biosafety and scientific issues of human gene therapy. Six proposals have been reviewed including studies of gene marking and gene transfer in diseases such as adenosine deaminase deficiency, cystic fibrosis, glioblastoma, and melanoma. In France, once a proposal is approved by this dual committee, the clinical trial can be initiated without further review by other agencies.

Dr. Walters remarked that recently a report has been published entitled: *Experimental (Somatic) Gene Therapy, Ethical Concerns and Controls* from Dr. M.A.M. de Wachter, Instituut voor Gezondheidsethiek, Maastrict, The Netherlands. This report surveyed human gene transfer studies and their oversight mechanisms in Europe. Once permission has been obtained from the publisher, copies will be made available to RAC members.

XV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *CLINICAL PROTOCOL FOR MODIFICATION OF ONCOGENE AND TUMOR SUPPRESSOR GENE EXPRESSION IN NON-SMALL CELL LUNG CANCER*/DR. ROTH

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Dr. Jack A. Roth of the MD Anderson Cancer Center, Houston, Texas. This protocol is a resubmission of a protocol that was contingently approved by the RAC at its September 1992 meeting. At its December 1993 meeting, the consensus of the RAC was that Dr. Roth should resubmit a revised protocol (including all additional data for review by the full RAC) based on the following: (1) failure of the primary reviewers to recommend approval of the protocol, (2) lengthy delays that occurred, (3) there are several new members on the RAC who were not on the committee at the time the original protocol was reviewed, and (4) Dr. Roth requested the use of a substitute vector. The RAC agreed that new primary reviewers would be assigned for the resubmitted protocol. The RAC informed Dr. Roth that this study was considered administratively inactivated; therefore, RAC approval of the protocol was withdrawn.

Dr. Motulsky explained that recent scientific advances have led to an understanding of oncogenes (i.e., K-*ras* and their role in tumor cell proliferation) and tumor suppressor genes (i.e., *p53* and their role in suppressing tumor growth). Although normal *p53* is a tumor suppressor gene, introduction of certain mutations can confer oncogenic capacity to *p53*. The investigator proposes to use the retroviral construct, AS-K-*ras*, to express antisense RNA in an attempt to block the function of the K-*ras* oncogene. Another construct, LN*p53*B, will be employed to express wild-type *p53* in an attempt to suppress tumor growth. Most of the preclinical animal experiments were performed with the retroviral construct LNSX-*p53*, which is different from the vector currently proposed for the human study. Additional studies have been submitted in a human lung tumor/nude mouse model demonstrating marked suppression in tumor growth in response to intrabronchial injection of the LNp53 construct. The RAC must consider the likelihood

that suppression of tumor growth in a murine model will correlate with the human response.

Dr. Motulsky stated that the RAC was previously concerned whether the constructs demonstrated biological activity and whether rearrangements in the vector structure are likely to occur during vector propagation. The Southern blot data submitted is uninterpretable. The principal investigator has adequately responded to previous concerns about the sensitivity of the assays for detecting the transforming potential of the proposed constructs. The data demonstrates that the assay system will provide adequate sensitivity.

Dr. Motulsky recommended approval of this study based on biologic plausibility; however, other reviewers' concerns about vector rearrangements, etc., must be addressed before full RAC approval can be recommended.

Review--Dr. Haselkorn (presented by Dr. Motulsky)

Dr. Motulsky summarized the written review submitted by Dr. Haselkorn. Non-small cell lung carcinoma has a very poor prognosis. Molecular analysis has revealed that mutations in the *p53* tumor suppressor gene and in the K-*ras* oncogene account for the majority of cases of this type of cancer. Therefore, therapy targeted to these genes seems justified. Patients will be eligible who have inoperable lung cancers, who are not responsive to radiation, and who demonstrate a high probability of dying from pneumonia caused by blockage of the lung by the tumor mass. Following surgical debulking of the tumor mass by bronchoscopy, the residual tumor will be injected with retroviral constructs that express either the wild-type *p53* gene or an antisense RNA to prevent translation of the mutated K-*ras* oncogene. The previous review raised three major issues: (1) the ability to detect transforming viruses, (2) demonstration of adequate biological activity, and (3) demonstration of the "bystander" effect in *in vitro* cell mixing experiments. The "bystander" effect on tumor growth was observed in animal experiments. The first two concerns have been satisfactorily addressed by the investigator. The mechanism of the "bystander" effect remains unknown; however, absence of knowledge about the mechanism should not prevent the protocol from being approved. The investigators should explain why the *p53* gene does not induce apoptosis in all cell lines.

Review--Ms. Grossman

Ms. Grossman raised several serious concerns regarding the vectors used in this protocol. How will quality assurance of the clinical grade retroviral constructs to be administered to patients be maintained? The Southern blot analysis of vector DNA, which demonstrates the absence of vector rearrangement, is uninterpretable. The size of the DNA bands are inconsistent with the LN*p53*B construct. She asked the investigator to respond to Dr. Miller's written comments that the LN*p53*B construct has the propensity to undergo rearrangement in vector structure during viral propagation due to the bidirectional SV40 polyadenylation signal.

Ms. Grossman stated that the "bystander" effect attributed to the present system is different from the "bystander" effect observed in the herpes simplex virus thymidine kinase/ganciclovir protocols. The biological mechanism of the latter phenomenon is more clearly understood.

Ms. Grossman said that although Dr. Roth is a reputable physician qualified to conduct a study on small-cell lung cancer, there are serious concerns about the molecular biology aspects of this proposal.

Other Comments

Dr. Post asked whether the sensitivity of the assay for transforming viruses is adequate since this issue was a major concern during the previous review. Ms. Grossman asked what construct was assayed for transforming virus. Dr. Motulsky asked the investigators to clarify exactly what constructs are proposed for the human study. In addition, Dr. Post noted that large volumes of vector supernatants will be administered to these patients. Will there be any effect of these oncogene and antioncogene vectors on normal cells?

Dr. Parkman inquired about what is the rate of transduction in the animal tumor model. How does the rate of transduction relate to the efficacy of suppressing tumor growth in animals? Are these data reproducible? Dr. Parkman asked Dr. Roth to address the issue of vector structure in the clinical grade supernatants.

Investigator Response--Dr. Roth

Responding to Dr. Post's question on the effect of normal human cells upon transduction, Dr. Roth said that vectors expressing either the antisense K-*ras* or the wild-type *p53* gene demonstrate no appreciable *in vitro* effect on the proliferation of normal fibroblasts unless the *p53* gene is expressed at an extremely high level.

Dr. Roth made a short presentation about his protocol with illustrations in an attempt to address several general questions. Dr. Roth said that the eligible patients must have bronchial obstruction that is untreatable with conventional therapy and have an expected survival of 4 to 6 months. The proposed treatment is intended to slow tumor growth rather than be curative. Following a biopsy, a determination will be made as to whether the tumor has K-*ras* or *p53* gene mutation. Based on this information, the appropriate construct will be administered. Following partial endoscopic resection, the tumor bed will be irrigated with vector supernatants daily for 5 days through a bronchoscope. This treatment will be repeated monthly.

Dr. Parkman noted that there is reproducible therapeutic effect in the animal studies. A maximal response is obtained at a multiplicity of infection of 5 retroviral particles per tumor cell, but the response decreases to one-half when the ratio is decreased to one-third. Dr. Parkman asked about the multiplicity of infection that would be expected in the clinical protocol. In the animal experiments, all tumor cells are in cycling and are

susceptible to viral integration in the 24 hour period of treatment. What percentage of cells in human tumors will be in cycling? Dr. Roth answered that cell cycling problems will be overcome by administering repeated injections. Since the bulk of the tumor will be removed by laser treatment, a multiplicity of infection of 5 viral particles to one tumor cell is achievable.

Responding to the question of the size of DNA fragments in the Southern blot analysis of cells transduced with the LN*p53*B vector, Dr. Roth said that digestion with the Kpn restriction enzyme should yield a fragment approximately 8.7 kb. Ms. Grossman questioned the validity of the Southern blot data submitted by Dr. Roth. This data shows that DNA fragments of the same size of 10.8 kb are detected in cells either transduced by the LNSX vector or by the LN*p53*B construct with gene insert. Dr. Roth said that he was not sure why the DNA fragment migrates aberrantly at this point. Dr. Straus said that the DNA fragment with the *p53* gene insert should not migrate at the same rate as the fragment obtained from the vector itself. Dr. Roth said that the present 1% gel analysis will not permit resolution of this difference. Drs. Straus, Post, and Ms. Grossman said that the size difference should be approximately 5.5 kb, and it should be resolved in this gel analysis. Ms. Grossman asked whether Northern blot analysis of vector RNA in transduced cells has been performed in order to determine whether there is any vector structure rearrangement. Dr. Roth referred to a letter by Dr. Harry Findlay of Emory University, Atlanta, Georgia, which states that the transduced *p53* expression was detectable in his Northern blot analysis. Ms. Grossman said that the data is not presented to answer her question on vector rearrangement. Ms. Grossman remarked that from her understanding of the situation that every time the principal investigators at Genetic Therapy, Inc., Gaithersburg, Maryland, have made a production run on this virus, they found vector rearrangement. Dr. Roth said that they are using a different vector construct and a different producer cell line. Ms. Grossman said that vector rearrangement is an important issue to be resolved before proceeding to human trials. She said that the data presented is not convincing.

Responding to questions about the "bystander" effect, Dr. Roth presented data reported in his published paper entitled: *A Retroviral Wild-type p53 Expression Vector Penetrates Human Lung Cancer Spheroids and Inhibits Growth by Inducing Apoptosis*, published in *Cancer Research* (Vol. 53, pp. 4129-4133, 1993). This data demonstrated that substances released from *p53*-induced apoptosis inhibit tumor cell growth. Dr. Roth said that very recent data suggested that this substance may be a protein molecule since its activity is destroyed by protease digestion. This observation may offer a possible explanation for the mechanism of the "bystander" effect observed in the animal model.

Dr. Roth said that he is unable to perform the cell mixing experiment to demonstrate that cells transduced with *p53* are capable of inhibiting the growth of untransduced cells. Cells transduced with the *p53* gene construct undergo apoptosis, and they cannot be established as a cell line.

Responding to Dr. Parkman's question on *in vivo* transduction efficiency, Dr. Roth presented data demonstrating approximately 60% transduction efficiency in an orthotopic

human lung cancer nude mouse model. These data has been subjected to biomathematics analysis with confidence intervals between 30 to 100%. Dr. Roth said that this number is unexpectedly high. Dr. Straus questioned the interpretation of this data. Some of the effect could be explained by the DNA copy number per cell since Dr. Roth assumed that every DNA copy represents a cell. Dr. Roth said that single copy integration has been observed in the cell line. Dr. Parkman said that 60% efficiency would not be surprising for the transduction of a cell line if 100% of cells are in cell cycling. What percentage of wild-type tumor cells are in cycling? Dr. Roth said that he does not know the answer to that question. Dr. Lang Chang, Institute of Biomedical Sciences, Academia Sinica, Taipei, from the audience questioned the interpretation of the data on transduction efficiency.

Dr. Roth said that the antitumor effect of the retroviral constructs on the tumor growth is reproducible and significant, regardless of the underlying mechanism. Ms. Grossman emphasized that precise knowledge about the proposed constructs is essential for human studies. The RAC should not recommend approval of a human trial if the vectors to be applied to humans are not adequately characterized. Again, Ms. Grossman raised questions on uncertainty regarding the size of DNA fragments from Kpn digests of vector transduced cells. Dr. Roth conceded that uncertainty resulted from the problem that the sequence of the vector is not presented, thus, he is unable to provide size information.

Dr. Straus said that the animal data is impressive and justifies the human study. However, Dr. Straus agreed with Ms. Grossman's comment that a complete sequence of the vector construct is essential, and data demonstrating the integrity of the vector structure is necessary in order to proceed with the human study. Dr. Post said that the mechanism of the "bystander" effect and transduction efficiency in the animal model are not major issues; however, the complete vector sequence and the Southern blot data on the vector structure are essential. Dr. Post expressed his dissatisfaction with the data regarding the size of the DNA fragments, the Southern blot, and generally the characterization of the vector structure.

Dr. Roth showed a DNA sequence of the vector in an attempt to address the question of the Kpn fragment size. Dr. Roth said that in this sequence, a large section of the actin promoter of the vector is not included. Dr. Post said that this missing sequence information appears to be the source of some of the uncertainty regarding the DNA fragment size. Dr. Post said that this missing information raises another question of whether there is another Kpn site within this actin promoter segment. Kpn was originally presumed by the principal investigator to be a single cut enzyme that digests the DNA at a single site in each of the two long terminal repeat regions of the vector. Dr. Roth said that an additional Kpn site is unlikely since only two predicted DNA fragments are generated by digestion with Kpn. Ms. Grossman questioned the data since the digests of the construct with the insert are the same as the vector by itself. Dr. Straus said that the gel experiment presented should be able to distinguish a size difference of 4 kb between the fragments from the vector and the construct with the insert according to the molecular size markers included in this experiment. Dr. Parkman said that the data presented is of such poor quality that it cannot be accepted as a basis

for protocol approval. A large segment of vector sequence not accountable is not acceptable.

Ms. Grossman emphasized that for approval a vector must be completely sequenced or a detailed restriction enzyme map provided. A Southern blot analysis of cells transduced with the antisense K-*ras* and *p53* retroviral constructs, and a comparison with the LNSX vector control will be required. In addition, the Northern blot data on vector transcript should be provided. Dr. Roth said that Northern blot analysis will be difficult for tumor RNA.

Dr. Motulsky stated that although the animal data is impressive, a full characterization of the vectors is essential for this protocol.

In terms of characterization of the vector structures regarding both retroviral constructs containing the antisense K-*ras* and *p53* genes, Drs. Post, Straus, and Ms. Grossman stated that the following information is necessary: (1) the complete DNA sequences of the vector constructs, (2) a detailed restriction enzyme digestion map of the constructs, and (3) a series of Southern blot analyses of vector DNA from cells transduced with the parental vectors versus constructs containing the gene inserts. Data should be obtained from several transduced cell lines demonstrating concordance with the restriction enzyme analysis and the restriction map.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Parkman to approve the protocol. Approval of the protocol is contingent on the review and approval of the following data for both the K-*ras* and *p53* retroviral constructs by the primary reviewers and Drs. Post and Straus: (1) complete vector sequences, including detailed restriction enzyme maps relevant to the LNSX backbone and the gene inserts; and (2) Southern blot analyses using several transduced cell lines, including cell lines transduced with the vector constructs with and without gene inserts, demonstrating concordance with the restriction enzyme analysis and sequence data. The motion to approve the protocol passed by a vote of 13 in favor, 0 opposed, and no abstentions.

XVI. CHAIR REPORT - OUTGOING MEMBERS

Dr. Walters noted that several members of the RAC have completed their term of service. He thanked Drs. Carmen, Hirano, Post, Geiduschek and Krogstad, and Ms. Grossman, for their dedication, expertise, and tireless efforts, which have contributed significantly to the advancement of human gene therapy.

XVII. REPORT FROM THE WORKING GROUP ON INFORMED CONSENT - AMENDMENTS TO PART I-D OF THE *POINTS TO CONSIDER*/DR. ZALLEN

Dr. Gary Ellis, Director of the NIH Office for Protection from Research Risks, recommended several avenues that should be pursued by the RAC with regard to the

"quality and content of Informed Consent documents into constructive changes in the informed consent process," specifically in relation to human gene transfer, during his oral presentation to the RAC on December 4, 1993, and in his memorandum dated December 23, 1993. Dr. Ellis recommended that the *Points to Consider* should be amended to introduce consistency in the Informed Consent document language.

Dr. Zallen, Chair of the RAC Working Group on Informed Consent, said that in order to reduce the frequent problem of inadequately prepared Informed Consent documents and because of the importance of informed consent issues in clinical research, a working group has been formed to amend Part I-D, Informed Consent, of the *Points to Consider.* The working group includes: Ms. Buc, Mr. Capron, Ms. Meyers, Drs. Krogstad, Motulsky, Secundy, and Straus.

Dr. Zallen said that frequently investigators do not adequately address informed consent questions in their preparation of the *Points to Consider.* Dr. Zallen provided two versions of the revised Part I-D: (1) the version drafted by the working group, and (2) a modified version incorporating modifications suggested by Mr. Capron. The first version attempts to separate the informed consent process from the Informed Consent document. Ms. Meyers recommended inclusion of a statement indicating that subject selection should be equitable. The second version has been modified according to Mr. Capron's suggestions to consolidate the consent process and consent document in a single section in the Part I-D.

Dr. Zallen said that questions on how informed consent is obtained from study subjects is very important considering the recent scandal involving radiation research performed during the 1940s and 1950s. She would prefer the first version to have the informed consent process separated from the document. The second version has incorporated a more simplified and polished language suggested by Mr. Capron.

Dr. Straus expressed his concern that it is difficult for principal investigators to describe very intimate personal interactions involved in the consent process. Dr. Parkman added that an effective informed consent process may vary depending on a particular disease to be treated and the ethnic background of a participating subject. Dr. Secundy stated that the informed consent process can be well written. There are trained individuals with expertise in this area who can deal with this process effectively, and the qualification of these experts can be evaluated from their curriculum vitae. Dr. Parkman disagreed on the need for another trained expert to obtain informed consent. Dr. Straus inquired what will be the qualification of such individuals. Dr. Secundy said that those are persons trained in bioethics, social work, and communication skill. Demonstrating sensitivity to these issues is becoming a requirement for submitting grant applications to the Ethical, Legal, and Social Implications Program of the National Center for Human Genome Research at NIH. Dr. Straus said that a well written Informed Consent document would indicate whether an effective informed consent would be obtained. Dr. Parkman expressed his reservation about separation of the process from the document in two sections. Dr. Secundy said that the informed consent process can be described in a short statement.

Dr. Walters said that it is not feasible to do detailed editing around the table about the revision, and he suggested focusing on the specific amendments contained in the Part I-D-2 regarding the Informed Consent document for the present discussion. Dr. Parkman said that Part I-D-2-b-(9), *Explanation to Participants of the Specific Requirements of Gene Transfer Research*, furnishes the Principal Investigators specific information on how the participants should be informed. If specific language for each required element is not written, the investigators may not satisfactorily address these elements in their Informed Consent document. Dr. Zallen said that these specific languages can be provided by the ORDA on a list apart from the *Points to Consider*. Dr. Parkman suggested that questions in Part I-D-2-b-(9) should be prefaced with an explanation as to the necessity for the requested information.

Dr. Straus said that Part I-D-2-b-(3) regarding possible risk, discomfort, and side effects should be written more explicitly to inform patients about risks in gene transfer studies. Dr. Straus will provide a sentence for this section. Dr. Secundy suggested inclusion of a statement to require the Informed Consent document should be written in a language understandable to laypersons. Dr. Walters suggested to condense Part I-D-1 through I-D-1-a-(2), and to rearrange sections dealing with patient selection, privacy and confidentiality, and special issues. Drs. Zallen and Secundy said that questions contained in these sections are designed to ensure that no conflict of interest or coercion is involved in the informed consent process. Dr. Walters suggested to revise the Part I-D-1-a on communication of the study with potential participants in a procedural manner. Dr. DeLeon suggested to preface those questions in terms of why the questions were asked.

The RAC recommended that the working group should develop a consolidated version of Part I-D, particularly Part I-D-2-b-(9), *Explanation to Participants of the Specific Requirements of Gene Transfer Research*, which includes language from both proposed documents. The RAC suggested that questions should be prefaced with an explanation as to the necessity for the requested information. Dr. Walters stated that since a broad consensus have been made on how to revise the informed consent section in Part I-D, the working group will be able to use these recommendations and submit a revised document to the next meeting.

XVIII. CONTINUATION OF THE RAC WORKING GROUP DISCUSSION ON ACCELERATED REVIEW - AMENDMENTS TO THE *NIH GUIDELINES* AND THE *POINTS TO CONSIDER*/DR. PARKMAN

Dr. Parkman presented an overview of the *Proposed Cover Sheet for Accelerated Review of Human Gene Transfer Experiments (Accelerated Review)*. The proposed cover sheet could be adopted as a preliminary mechanism for screening human gene transfer studies that may qualify for the accelerated review process previously approved by the RAC. Principal Investigators requesting consideration of their protocol for the *Accelerated Review* process must complete the cover sheet. Based on the information provided by the Principal Investigator, NIH/ORDA in consultation with the RAC Chair and one or more RAC members, as necessary, will make a determination regarding eligibility for

Accelerated Review. In the event that a protocol is denied review by this accelerated process, the proposal will be reviewed by the full RAC.

Dr. Smith said that the text on the proposed definition of the category on "umbrella" protocols need to be clarified. The category is not intended for a Principal Investigator to initiate a new study at multiple sites without a major review. Ms. Wilson explained that the definition applies to protocols in which the Principal Investigators indicated in their submission that the studies will be performed at multiple sites. Dr. Smith asked whether the initial review will be a *Major Action*. Dr. Parkman said that the initial review is a *Major Action*. Ms. Grossman asked whether the multicenter trial proposed by Vical, Inc., San Diego, California, that is based on a protocol previously approved for other Principal Investigators will be included in this category. Dr. Parkman explained that this trial involves different study designs at many sites and is not a typical "umbrella" protocol. For reviewing an "umbrella" protocol, the master protocol will receive a major review and will be considered a *Major Action* when first submitted. When the Principal Investigator requests inclusion of additional sites, these modifications will be reviewed as a *Minor Action*. Ms. Grossman asked whether this category would include a protocol wherein a new Principal Investigator intends to perform a previously approved protocol at additional sites. Dr. Parkman said that if nothing substantially new is proposed, it could qualify as a *Minor Action*. Dr. Walters asked if the "umbrella" protocols differ from other categories involving new Principal Investigators and new sites. Dr. Parkman cited as a best example of the "umbrella" protocol would be the study of brain tumor treatment with herpes simplex virus thymidine kinase/Ganciclovir in which standard virus producer cells will be supplied from a central laboratory for administration to patients at multiple sites. Dr. Parkman said that the "umbrella" category is new and that no protocols have been proposed for RAC review. Dr. Parkman noted that there is no big difference from other categories dealing with new sites and new Principal Investigators, but he envisioned that repeated submission of new protocol documents will not be needed in this "umbrella" category.

Dr. Straus asked to clarify the definition of the Category 3 on new site/original Principal Investigator. Dr. DeLeon said that this category will be for a Principal Investigator to transfer a RAC-approved protocol to a new site. Dr. Zallen asked whether this request will be a minor modification. Dr. Wivel remarked that minor modification is also a *Minor Action* similar to *Accelerated Review*. Dr. Parkman explained that the reason to create this category for *Accelerated Review* is that transfer of a protocol to a new site sometimes will involve additional issues such as new laboratory expertise and new personnel. These new issues will be reviewed with a new protocol submission. Dr. Straus stated his understanding that Category 4, new site/new Principal Investigator, will be for a Principal Investigator who wishes to initiate a protocol previously approved by the RAC at a new site, and Category 3, new site/original Principal Investigator, will be for a Principal Investigator to take his or her own RAC-approved protocol to a new site.

Dr. Post suggested that Dr. Parkman and the ORDA develop the final language to define these categories. Dr. Motulsky concurred.

Dr. Parkman introduced the checklist to be submitted by the Principal Investigators with their protocols for *Accelerated Review*. The purpose of the checklist is to have the Principal Investigators answer the pertinent questions in order to aid the ORDA staff in determining eligibility for *Accelerated Review*. The checklist is very similar to the one approved for the single-patient *Expedited Review* except for item B-11. Item B-11 asks the question whether the vector has been reviewed and approved for clinical investigation by the Food and Drug Administration. It is certain that the vector will be available for single-patient expedited trial, and the question is not relevant for the present *Accelerated Review*. Dr. Parkman suggested the deletion of this item from the proposed check list.

Ms. Grossman asked to clarify item C-4 on the question of whether the proposed study is similar to another RAC-approved protocol. The Principal Investigator needs to identify the major differences in the checklist.

Dr. Secundy asked for clarification of item E dealing with the Informed Consent document. Dr. Parkman explained that these listed elements for Informed Consent are frequently overlooked by the Principal Investigators, and the listing is to assure that the Principal Investigators have included these elements. Dr. Parkman said that submission for *Accelerated Review* should be a complete document similar to that required for regular review including an IRB-approved Informed Consent document, *Points to Consider*, etc. Drs. Straus and Zallen agreed with Dr. Parkman that a listing of required Informed Consent elements will aid the ORDA staff in determining whether all important questions have been addressed by the Principal Investigators in their Informed Consent. Dr. Secundy noted that the language should be clarified to indicate that these elements are essential but not the only required elements for the Informed Consent document. Dr. Straus said that these additional elements are already incorporated into the revised *Points to Consider*. Dr. Parkman explained that the purpose of highlighting these elements is to aid ORDA staff in determining whether they are addressed by the Principal Investigators. Dr. Straus suggested language to ask Principal Investigators to provide a copy of the IRB-approved Informed Consent document, consistent with the *Points to Consider*, and to underline the text addressing the listed specific items. Dr. Zallen remarked that there are circumstances in which a particular element is not appropriate for inclusion in a particular Informed Consent document. She cited an example where the Principal Investigators might avoid mentioning an autopsy in the Informed Consent document to be prepared for children. Dr. Zallen said that if a particular item is not addressed in the Informed Consent document, the Principal Investigators should provide an explanation as to the reason for its omission.

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Secundy to accept the proposed cover sheet with the incorporation of minor modifications by a vote of 13 in favor, 0 opposed, and no abstentions. The current document will be divided into 2 separate documents: (1) *Accelerated Review* cover sheet, and the (2) *Cover Sheet for Expedited Review of a Single Patient Human Gene Transfer Experiment (Expedited Review)*. The difference between these two documents is that the *Expedited Review* cover sheet will include the following addition questions: "Has the vector been reviewed and

accepted for clinical investigation by the FDA? What was the date of IND submission? Is there a sufficient supply of the clinical grade material available to complete the proposed study? If clinical grade material is unavailable, on what date will such material become available?" These additional questions that will be incorporated into the *Expedited Review* cover sheet will be included as item number 11 under Section B, *Vector, Target Cell, and Transduction Procedures*.

The RAC-approved version of the *Accelerated Review* cover sheet reads:

PROPOSED COVER SHEET FOR ACCELERATED REVIEW OF A HUMAN GENE TRANSFER EXPERIMENT

A. BACKGROUND

1. Provide the following information about the proposed study: title, principal investigators, and participating institutions.

B. VECTOR, TARGET CELL, AND TRANSDUCTION PROCEDURES

1. What are the proposed gene and vector for this protocol?
2. Has the proposed vector previously been approved by the RAC? If so, provide the title and principal investigator(s) of at least one RAC-approved protocol utilizing this vector. If not, attach the complete vector sequence (hard copy and a 3½ inch diskette in ASCII format).
3. Who is the vector supplier?
4. What is the target cell to be transduced by the proposed vector?
5. What is the rate of transduction of the proposed vector and target cells in the proposed setting (i.e., your laboratory)?
6. What is the level of gene expression demonstrated in the target cell?
7. How was gene expression determined?
8. What assay was used to detect replication-competent virus (RCR)?
9. Was RCR detected?
10. What is the level of sensitivity of the RCR assay? (Attach documentation).

C. CLINICAL PROTOCOL

1. What in vitro or in vivo system(s) were used to determine preclinical efficacy?
2. What is the end point of the protocol?
3. Is the proposed study identical to another RAC-approved protocol except that the study will be performed at a satellite institution? If so, provide a letter of cross-reference from the sponsoring institution.
4. Is the proposed study similar to another RAC-approved protocol? Identify the major differences.

D. **LOCAL COMMITTEE APPROVALS**

1. Has the proposed study been unconditionally approved by your Institutional Biosafety Committee? If so, provide a copy of the approval.

2. Has the proposed study been unconditionally approved by your Institutional Review Board? If so, provide a copy of the approval.

E. **INFORMED CONSENT DOCUMENT**

1. Provide a copy of the IRB-approved Informed Consent document, consistent with the Points to Consider, and underline the text addressing the following specific items:

NOTE: If any of these items are not addressed in the Informed Consent document, provide an explanation as to the reason for their omission.

a. Any requirement for use of birth-control by male and female participants during the course of the experiment;

b. Financial costs for which the individual research subject will be responsible;

c. Need for long-term follow-up and the arrangements for such follow-up;

d. Statement indicating that a request for permission to perform an autopsy will be made of the family, regardless of the immediate cause of death;

e. Arrangements in place at the research institution for sharing information with the new media and the public;

f. Provisions for protecting patient privacy and the confidentiality of data obtained from individual participants in the research.

F. **ADDITIONAL INFORMATION**

1. Provide curricula vitae (2 page Biosketch format) for principal investigators and key personnel.

2. Provide relevant publications only.

The RAC-approved version of the *Cover Sheet for Expedited Review of a Single Patient Human Gene Transfer Experiment (Expedited Review)* cover sheet reads:

COVER SHEET FOR EXPEDITED REVIEW
OF A SINGLE PATIENT HUMAN GENE TRANSFER EXPERIMENT
(Expedited Review)

A. BACKGROUND

1. Provide the following information about the proposed study: title, principal investigators, and participating institutions.

B. VECTOR, TARGET CELL, AND TRANSDUCTION PROCEDURES

1. What are the proposed gene and vector for this protocol?
2. Has the proposed vector previously been approved by the RAC? If so, provide the title and principal investigator(s) of at least one RAC-approved protocol utilizing this vector. If not, attach the complete vector sequence (hard copy and a 3½ inch diskette in ASCII format).
3. Who is the vector supplier?
4. What is the target cell to be transduced by the proposed vector?
5. What is the rate of transduction of the proposed vector and target cells in the proposed setting (i.e., your laboratory)?
6. What is the level of gene expression demonstrated in the target cell?
7. How was gene expression determined?
8. What assay was used to detect replication-competent virus (RCR)?
9. Was RCR detected?
10. What is the level of sensitivity of the RCR assay? (Attach documentation).
11. Has the vector been reviewed and accepted for clinical investigation by the Food and Drug Administration? What was the date of investigational new drug (IND) submission? Is there a sufficient supply of the clinical grade material available to complete the proposed study? If clinical grade material is unavailable, on what date will such material become available?

C. CLINICAL PROTOCOL

1. What in vitro or in vivo system(s) were used to determine preclinical efficacy?
2. What is the end point of the protocol?
3. Is the proposed study identical to another RAC-approved protocol except that the study will be performed at a satellite institution? If so, provide a letter of cross-reference from the sponsoring institution.
4. Is the proposed study similar to another RAC-approved protocol? Identify the major differences.

D. LOCAL COMMITTEE APPROVALS

1. Has the proposed study been unconditionally approved by your Institutional Biosafety Committee? If so, provide a copy of the approval.

2. Has the proposed study been unconditionally approved by your Institutional Review Board? If so, provide a copy of the approval.

E. INFORMED CONSENT DOCUMENT

1. Provide a copy of the IRB-approved Informed Consent document, consistent with the Points to Consider, and underline the text addressing the following specific items:

NOTE: If any of these items are not addressed in the Informed Consent document, provide an explanation as to the reason for their omission.

a. Any requirement for use of birth-control by male and female participants during the course of the experiment;

b. Financial costs for which the individual research subject will be responsible;

c. Need for long-term follow-up and the arrangements for such follow-up;

d. Statement indicating that a request for permission to perform an autopsy will be made of the family, regardless of the immediate cause of death;

e. Arrangements in place at the research institution for sharing information with the new media and the public;

f. Provisions for protecting patient privacy and the confidentiality of data obtained from individual participants in the research.

F. ADDITIONAL INFORMATION

1. Provide curricula vitae (2 page Biosketch format) for principal investigators and key personnel.

2. Provide relevant publications only.

XIX. CLOSING REMARKS

Dr. Walters asked the RAC members if there were any outstanding issues that should be addressed prior to adjournment of this meeting. Dr. Hirano questioned the time-frame from publication of an incorporated version of the *NIH Guidelines*. Dr. Wivel responded that such document is in final format and is awaiting completion of the pending environmental assessment for Appendices P and Q.

Dr. Motulsky said that the RAC has extensively discussed the informed consent issues related to gene therapy. These are very important issues in clinical medical research. If the conclusion of this discussion is published in a research journal, it will benefit investigators in other areas of clinical research and will save the RAC a great deal of time without having to revisit the same issue in deliberation of each protocol.

Dr. Parkman said that the RAC should continue to solicit *ad hoc* experts who could provide relevant presentations regarding inadvertent germ-line transmission. Such presentations would represent a continuation of series of educational presentations on the issue of germ-line gene transfer. With the advent of genetic science, the development of the framework and policy for reviewing such protocols is important. Dr. Walters said that it is an excellent suggestion and the Institute of Medicine of the National Academy of Sciences is proposing to initiate a formal study on gene intervention in germ-line cells. Dr. Post said that the immediate likelihood of the RAC to encounter this problem is the inadvertent germ-line modification in gene transfer studies.

Dr. Walters stated that Dr. Harold Varmus, NIH Director, has indicated his intention to attend the September 1994 RAC meeting.

XX. ADJOURNMENT

Dr. Walters adjourned the meeting at 2:32 p.m. on March 4, 1994.

Nelson A. Wivel

Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: 6/7/94

LeRoy B. Walters

LeRoy B. Walters, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health

RECOMBINANT DNA ADVISORY COMMITTEE

CHAIR

WALTERS, LeRoy B., Ph.D. (95)
Director, Center for Bioethics
Kennedy Institute of Ethics
Georgetown University
1437 37th St., N.W., Room 215
Washington, D.C. 20057
202 687-6771

BRINCKERHOFF, Constance E., Ph.D. (94)
Professor
Department of Medicine
Dartmouth Medical School
2 Maynard Street
Hanover, New Hampshire 03755
603 650-1609

BUC, Nancy L., LL.B. (94)
Partner
Buc, Levitt, & Beardsley
1800 M Street, N.W.
Washington, D.C. 20036
202 736-3610

CAPRON, Alexander M., LL.B. (94)
Professor
The Law Center
University of Southern California
Los Angeles, California 90089-0071
213 740-2557

CARMEN, Ira H., Ph.D. (93)
Professor
Department of Political Science
University of Illinois
361 Lincoln Hall, 702 S. Wright St.
Urbana, Illinois 61801-3696
217 333-3881

CHASE, Gary A., Ph.D. (95)
Professor, Dept. of Mental Hygiene
The Johns Hopkins University
624 N. Broadway, Room 855
Baltimore, Maryland 21205
410 955-3991

DeLEON, Patricia A., Ph.D. (95)
Professor
Department of Human Genetics
School of Life and Health Sciences
University of Delaware, 219 McKinly
Newark, Delaware 19716
302 831-2249

DOI, Roy H., Ph.D. (94)
Professor, Section of Mol. & Cell. Biology
Division of Biological Sciences
University of California
Davis, California 95616
916 752-3191

DRONAMRAJU, Krishna R., Ph.D. (95)
President
Foundation for Genetic Research
P.O. Box 27701-497
Houston, Texas 77227
713 961-0415

GEIDUSCHEK, E. Peter, Ph.D. (93)
Professor
Center for Molecular Genetics, M-034
University of California, San Diego
La Jolla, California 92093-0634
619 534-3029 or 2451

GROSSMAN, Mariann (93)
Director, Human Applications Lab
Institute for Human Gene Therapy
Hospital of the Univ. of Pennsylvania
6 Maloney Bldg., 36th & Spruce Streets
Philadelphia, Pennsylvania 19104
215 349-8600

HASELKORN, Robert, Ph.D. (94)
Professor
Department of Molecular Genetics and Cell Biology
University of Chicago, 920 E. 58th St.
Chicago, Illinois 60637
312 702-1069 or 1620

HIRANO, Susan S., Ph.D. (93)
Associate Scientist
Department of Plant Pathology
University of Wisconsin
1630 Linden Drive
Madison, Wisconsin 53706
608 262-7236

KROGSTAD, Donald J., M.D. (93)
Henderson Professor and Chairman
Department of Tropical Medicine
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, Louisiana 70112
504 588-5199

MEYERS, Abbey S. (96)
Executive Director
National Organization for Rare Disorders
P.O. Box 8923
New Fairfield, Connecticut 06812
203 746-6518

MILLER, A. Dusty, Ph.D. (95)
Associate Member
Program in Molecular Medicine
Fred Hutchinson Cancer Research Cntr.
1124 Columbia Street
Seattle, Washington 98104
206 667-2855

MOTULSKY, Arno G., M.D. (96)
Professor
Department of Medicine & Genetics
University of Washington Medical School
RG-25
Seattle, Washington 98195
206 543-3593

PARKMAN, Robertson, M.D. (95)
Head, Div. of Research Immunology--Bone Marrow Transplantation
Childrens Hospital of Los Angeles
4650 Sunset Boulevard
Los Angeles, California 90027
213 669-2546

POST, Leonard E., Ph.D. (93)
Vice President
Experimental Therapy
Parke-Davis Pharmaceutical Division
2800 Plymouth Road
Ann Arbor, Michigan 48105
313 996-2899

SECUNDY, Marian G., Ph.D. (96)
Professor, Dept. of Community Health and Family Practice
Howard University College of Medicine
520 W Street, N.W.
Washington, D.C. 20059
202 806-6397

SMITH, Brian R., M.D. (96)
Associate Professor of Laboratory Medicine
Internal Medicine & Pediatrics
Yale University School of Medicine
333 Cedar Street
New Haven, Connecticut 06510-3333
203 785-2286

STRAUS, Stephen E., M.D. (96)
Chief
Laboratory of Clinical Investigation, NIAID
National Institutes of Health
Building 10, Room 11N228
Bethesda, Maryland 20892
301 496-5221

ZALLEN, Doris T., Ph.D. (95)
Associate Professor
Science Studies and Humanities
VA Polytechnic Inst. & State University
Humanities Center, Lane Hall-354
Blacksburg, Virginia 24061
703 231-4216

EXECUTIVE SECRETARY

WIVEL, Nelson A., M.D.
Director
Office of Recombinant DNA Activities
National Institutes of Health, 31/4B11
Bethesda, Maryland 20892
301 496-9838

NON-VOTING AGENCY REPRESENTATIVES

DEPARTMENT OF AGRICULTURE (USDA)

TOLIN, Sue A., Ph.D.
Science and Education
Cooperative State Research Service
USDA, Suite 330, Aerospace Bldg.
Washington, D.C. 20250-0220
202 447-5741

GAY, Cyril G., Ph.D. (ALT)
Veterinary Biologics
USDA, Federal Building, Room 836
6505 Belcrest Road
Hyattsville, MD 20782
301 436-8674

DEPARTMENT OF COMMERCE

GREIFER, Bernard, Ph.D.
Economics & Statistics Administration
Office of Policy Analysis
Room H-4858
Department of Commerce
Washington, D.C. 20230
202 482-3078

SHYKIND, Edwin, Ph.D. (ALT)
Trade Development, Room H-4045
International Trade Administration
Department of Commerce
Washington, D.C. 20230
202 377-4694

DEPARTMENT OF ENERGY

DRELL, Daniel W., Ph.D.
Office of Health and Environmental Research
Department of Energy, ER-72
Washington, D.C. 20545
301 903-4742

DEPARTMENT OF HEALTH AND HUMAN SERVICES (DHHS)

DHHS Alcohol, Drug Abuse and Mental Health Administration

GAUSE, Emily M.
Office of the Science Advisor
Alcohol, Drug Abuse and Mental Health Administration
5600 Fishers Lane, Room 1295
Rockville, Maryland 20857
301 443-4335

AUTRY, Joseph H., III, M.D. (ALT)
Office of Policy Coordination
Alcohol, Drug Abuse and Mental Health Administration
5600 Fishers Lane, Room 12C-06
Rockville, Maryland 20857
301 443-4111

DHHS Centers for Disease Control (CDC)

GUINAN, Mary E., M.D., Ph.D.
Office of the Director
Centers for Disease Control
1600 Clifton Road, D22
Atlanta, Georgia 30333
404 639-3701

DHHS, CDC National Institute for Occupational Safety and Health

LEMEN, Richard A.
National Institute for Occupational
Safety and Health
4676 Columbia Parkway
Cincinnati, Ohio 45226
513 684-8302

DHHS Food and Drug Administration

WOODCOCK, Janet, M.D.
Office of Therapeutic Research & Review
Center for Biologics, Evaluation & Res.
FDA, Woodmont Suite 400S
1401 Rockville Pike
Rockville, Maryland 20852-1448
301 594-1106

GUNTER, Kurt C., M.D. (ALT)
Division of Cellular and Gene Therapy
Food and Drug Administration, HFM-515
1401 Rockville Pike
Rockville, Maryland 20852-1448
301 402-1336

DHHS Health Resources and Services Administration

MANLEY, Audrey, M.D.
Division of Health Service Corps
Administration
Health Resources & Services Administration
5600 Fishers Lane, Room 7A39
Rockville, Maryland 20857
301 443-2900

DHHS Office of the Assistant Secretary for Health

HUDSON, Kathy
Office of Health Planning and Evaluation
DHHS Office of the Assistant Secretary
for Health
200 Independence Avenue, S.W., Room 740G
Washington, D.C. 20201
202 690-8033

DEPARTMENT OF THE INTERIOR

PIMENTEL, Mariano B., M.D.
Division of Medical & Health Services
Department of the Interior, Room 7045
18th & C Street, N.W.
Washington, D.C. 20240
202 343-2081

DEPARTMENT OF LABOR

YODAIKEN, Ralph E., M.D.
Senior Medical Advisor
USDOL/OSHA, Room N-3653
200 Constitution Avenue, N.W.
Washington, D.C. 20210
202 523-7047

DEPARTMENT OF STATE

SAVAGE, Eleanor W.
Office of Ecology, Health & Conservation
OES/EHS, Room 4325
Department of State
2201 C Street, N.W.
Washington, D.C. 20520
202 647-2418

WEST, Peter A., M.D. (ALT)
Office of Ecology, Health & Conservation
OES/EHC, Room 4325
Department of State
2201 C Street, N.W.
Washington, D.C. 20520
202 647-2418

DEPARTMENT OF TRANSPORTATION

CUSHMAC, George E., Ph.D.
Research & Special Program Administration
Department of Transportation
400 7th Street, S.W.
Washington, D.C. 20590
202 426-2311

ENVIRONMENTAL PROTECTION AGENCY

FREDERICK, Robert J., Ph.D.
Office of Environmental Processes
and Effects Research, RD-682
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
202 382-5989

MILEWSKI, Elizabeth, Ph.D. (ALT)
Office of Pesticides & Toxic Substances
Environmental Protection Agency, RD-682
401 M Street, S.W.
Washington, D.C. 20460
202 382-2914

NATIONAL SCIENCE FOUNDATION

HARRIMAN, Phillip, Ph.D.
Division of Molecular Biosciences
National Science Foundation, Room 325
Washington, D.C. 20550
202 357-9687

LIAISON REPRESENTATIVES

TAKAGI, Yasuyuki, M.D.
Division of Molecular Genetics
Fujita Gakuen Health University
Toyoake, Aichi, 470-11
JAPAN
(0562) 93-9375

JONES, Daniel P., Ph.D.
Humanities, Science and Technology
Division of Research Programs
National Endowment for the Humanities
Washington, D.C. 20506
202 786-0207

FAYL, Gilbert
Counselor, Science & Technology Section
Delegation of the Commission of the
European Communities
2100 M Street, N.W., 7th Floor
Washington, D.C. 20037
202 862-9575

HUMAN GENE TRANSFER PROTOCOLS

8810-001[a] (M) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2 and Tumor Infiltrating Lymphocytes.*
RAC Approval: 10-3-88/NIH Approval: 3-2-89
Minor Modification: 3-30-90

9007-002[a,b] (T) Blaese, R. Michael, National Institutes of Health, Bethesda, Maryland; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells*
RAC Approval: 7-31-90/NIH Approval: 9-6-90
Major Amendment/RAC Approval: 2-10-92
Major Amendment/NIH Approval: 4-22-92
Minor Modification: 4-21-93
Minor Modification: 6-7-93

9007-003[a] (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor.*
RAC Approval: 7-31-90/NIH Approval: 9-6-90
Minor Modification: 2-17-94

9102-004[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Hurwitz, Craig; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 2-4-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-005[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-006[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-007[a] (M) Deisseroth, Albert B., M.D. Anderson Cancer Research Center, Houston, Texas; *Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 4-19-93

9105-008 (M) Ledley, Fred D.; Woo, Savio; Ferry, George; and Hartwell, Whigennand, Baylor College of Medicine, Houston, Texas; *Hepatocellular Transplantation in Acute Hepatic Failure and Targeting Genetic Markers to Hepatic Cells.*
RAC Approval: 5-30-91/NIH Approval: 7-12-91

9105-009[a] (M) Lotze, Michael T., University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; *The Administration of Interleukin-2 and Tumor Infiltrating Lymphocytes to Patients with Melanoma.*
RAC Approval: 5-30-91/NIH Approval: 1-17-92
Minor Modification: 11-30-92

9110-010[a] (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91
Minor Modification: 7-9-92

9110-011 (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91

9110-012[a] (T) Wilson, James M., University of Pennsylvania, Philadelphia, Pennsylvania; *Ex Vivo Gene Therapy of Familial Hypercholesterolemia.*
RAC Approval: 10-8-91/NIH Approval: 11-14-91
Minor Modification: 12-3-92
Minor Modification: 8-18-93

9202-013[a] (T) Nabel, Gary J., University of Michigan, Ann Arbor, Michigan; *Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors.*
RAC Approval: 2-10-92/NIH Approval: 4-17-92
Minor Modification: 1-22-93
Minor Modification: 1-93

9202-014 (M) Cornetta, Kenneth, Indiana University, Indianapolis, Indiana; *Retroviral-Mediated Gene Transfer of Bone Marrow Cells during Autologous Bone Marrow Transplantation for Acute Leukemia.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-015 (M) Economou, James S. and Belldegrun, Arie, University of California at Los Angeles, Los Angeles, California; *The Treatment of Patients with Metastatic Melanoma and Renal Cell Cancer Using In Vitro Expanded and Genetically-Engineered (Neomycin Phosphotransferase) Bulk, CD8 (+) and/or CD4(+) Tumor Infiltrating Lymphocytes and Bulk, CD8(+) and/or CD4(+) Peripheral Blood Leukocytes in Combination with Recombinant Interleukin-2 Alone, or with Recombinant Interleukin-2 and Recombinant Alpha Interferon.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-016[a] (T) Freeman, Scott M., Tulane University Medical Center, New Orleans, Louisiana; *Gene Transfer for the Treatment of Cancer.*
RAC Approval: 2-10-92/NIH Approval: 2-5-93
Minor Modification: 8-6-93

9202-017[b] (M) Greenberg, Philip D. and Riddell, Stanley, Fred Hutchinson Cancer Research Center, University of Washington, Seattle; *Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Genetically Modified CD8+ HIV-Specific T Cells in HIV Seropositive Individuals.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92
Major Amendment/RAC Approval: 9-9-93
Major Amendment/NIH Approval: 10-7-93

9206-018 (T) Brenner, Malcolm K.; Furman, Wayne; Santana, Victor; Bowman, Laura; and Meyer, William, St. Jude Children's Research Hospital, Memphis, Tennessee; *Phase I Study of Cytokine-Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92

9206-019 (T) Oldfield, Edward, National Institutes of Health, Bethesda, Maryland; *Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92
Minor Modification: 11-29-93

9206-020[a] (M) Deisseroth, Albert B., MD Anderson Cancer Center, Houston, Texas; *Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 4-19-93

9206-021 (T) Gansbacher, Bernd; Houghton, Alan; and Livingston, Philip, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with HLA-A2 matched Allogeneic Melanoma Cells that Secrete Interleukin-2 in Patients with Metastatic Melanoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9206-022[a] (T) Gansbacher, Bernd; Motzer, Robert; Houghton, Alan; and Bander, Neil, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 3-31-93

9206-023[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Multiple Myeloma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94

9206-024[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Breast Cancer.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94

9206-025[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94

9209-026 (M) Walker, Robert E., National Institutes of Health, Bethesda, Maryland; *A Study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in HIV Infected Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 9-3-93

9209-027[a] (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Phase I/II Study of PIXY321, a rhGM-CSF/rhIL-3 Fusion Protein, for the Mobilization of Peripheral Blood Stem Cells for Autologous Stem Cell Transplantation.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93
Minor Modification: 2/25/94

9209-028 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93
Protocol Closed: 2-25-94 (merged with protocol # 9209-027)

9209-029 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Trial of G-CSF Stimulated Peripheral Blood Stem Cells for Engraftment in Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93

9209-030 (M) Deisseroth, Albert B., University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients.*
RAC Approval: 9-14-92/NIH Approval: 12-2-93
Minor Modification: 11-3-93
Minor Modification: 11-18-93

9209-031 (T) Roth, Jack A., The University of Texas MD Anderson Cancer Center, Houston, Texas; *Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer.*
RAC Approval: 9-15-92/RAC Approval Withdrawn 12-3-93

9209-032 (M) Brenner, Malcolm K., St. Jude Children's Research Hospital, Memphis, Tennessee; A *Phase II Trial of the Baxter Neuroblastoma Bone Marrow Purging System Using Gene Marking to Assess Efficacy.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9209-033 (T) Lotze, Michael T. and Rubin, Joshua T., University of Pittsburgh, Pittsburgh, Pennsylvania; *Gene Therapy of Cancer: A Pilot Study of IL-4 Gene Modified Antitumor Vaccines.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9212-034[a] (T) Crystal, Ronald G., National Institutes of Health, Bethesda, Maryland; *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.*
RAC Approval: 12-3-92/NIH Approval: 4-16-93
Minor Modification: 5-17-93
Minor Modification: 10-8-93
Minor Modification: 11-29-93
Minor Modification: 2-4-94

9212-035[a] (T) Wilson, James M., University of Pennsylvania, Philadelphia, Pennsylvania; *Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial.*
RAC Approval: 12-3-92/NIH Approval: 8-26-93
Minor Modification: 8-17-93

9212-036 (M) Welsh, Michael J., Howard Hughes Medical Institute, Iowa City, Iowa, and Smith, Alan E., Genzyme Corporation, Framingham, Massachusetts; *Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium.*
RAC Approval: 12-4-92/NIH Approval: 4-16-93
Minor Modification: 10-18-93

9303-037 (T) Culver, Kenneth W., Iowa Methodist Medical Center, Des Moines, Iowa, and Van Gilder, John C.; University of Iowa, Iowa City, Iowa; *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.*
RAC Approval: 3-1-93/NIH Approval: 4-16-93

9303-038 (M) Heslop, Helen E.; Brenner, Malcolm K.; and Rooney, Cliona, St. Jude Children's Research Hospital, Memphis, Tennessee; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-039 (M) Brenner, Malcolm K.; Krance, Robert; Heslop, Helen E.; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-040 (T) Simons, Jonathan, Johns Hopkins Oncology Center, Baltimore, Maryland; *Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.*
RAC Approval: 3-1-93/NIH Approval: 12-2-93

9303-041 (T) Wilmott, Robert W. and Whitsett, Jeffrey, Children's Hospital Medical Center, Cincinnati, Ohio, and Trapnell, Bruce; Genetic Therapy, Inc., Gaithersburg, Maryland; *A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-042* (T) Boucher, Richard C. and Knowles, Michael R., University of North Carolina, Chapel Hill, North Carolina; *Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity.*
RAC Approval: 3-2-93/NIH Approval: 10-7-93
Minor Modification: 2-17-94

9306-043 (T) Seigler, Hilliard F., Duke University Medical Center, Durham, North Carolina; *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-044* (T) Deisseroth, Albert B.; Kavanagh, John; and Champlin, Richard, University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.*
RAC Approval: 6-7-93/NIH Approval: 12-2-93
Minor Modification: 2-25-94
Minor Modification: 2-25-94

9306-045 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-046 (T) Barranger, John A., University of Pittsburgh, Pittsburgh, Pennsylvania, *Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34(+) Cells.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-047 (T) Karlsson, Stefan and Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland, and Kohn, Donald B., Childrens Hospital Los Angeles, Los Angeles, California; *Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-048 (T) Galpin, Jeffrey E., University of Southern California, and Casciato, Dennis A., University of California, Los Angeles, California; *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-049 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *A Molecular Genetic Intervention for AIDS - Effects of a Transdominant Negative Form of Rev.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-EXP (T) Sobol, Robert and Royston, Ivor, San Diego Regional Cancer Center, San Diego, California; *CONFIDENTIAL.*
NIH Approval: 12-28-92
Major Amendment (Expedited Review)/NIH Approval: 5-11-93

9306-050 (T) Raffel, Corey, Childrens Hospital Los Angeles, Los Angeles, California, and Culver, Kenneth, Iowa Methodist Medical Center, Des Moines, Iowa; *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-051 (T) Hesdorffer, Charles and Antman, Karen, Columbia University College of Physicians and Surgeons, New York, New York; *Human MDR Gene Transfer in Patients with Advanced Cancer.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-052 (T) Ilan, Joseph, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*
RAC Approval: 6-8-93/NIH Approval: 12-2-93

9309-053 (T) Cassileth, Peter; Podack, Eckhard R.; Sridhar, Kasi; University of Miami, and Savaraj, Niramol; Miami Veterans Administration Hospital, Miami, Florida; *Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small Cell Lung Cancer.*
RAC Approval: 9-9-93/NIH Approval: 12-2-93

9309-054 (T) O'Shaughnessy, Joyce; National Institutes of Health, Bethesda, Maryland; *Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR-1) into Hematopoietic Stem Cells During Autologous Transplantation after Intensive Chemotherapy for Breast Cancer.*
RAC Approval: 9-9-93/NIH Approval: 10-7-93

9309-055 (T) Kun, Larry E.; Sanford, R. A.; Brenner, Malcolm K.; and Heideman, Richard L.; St. Jude Childrens Research Hospital, Memphis, Tennessee; and Oldfield, Edward H.; National Institutes of Health, Bethesda, Maryland; *Gene Therapy for Recurrent Pediatric Brain Tumors.*
RAC Approval: 9-9-93/NIH Approval: 10-7-93

9309-056 (T) Das Gupta, Tapas K. and Cohen, Edward P.; University of Illinois at Chicago; Chicago, Illinois; *Immunization of Malignant Melanoma Patients with Interleukin 2-Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.*
RAC Approval: 9-10-93

9309-057 (T) Wong-Staal, Flossie; Poeschla, Eric; and Looney, David; University of California, San Diego, California; *A Phase I Clinical Trial to Evaluate the Safety and Effects in HIV-1 Infected Humans of Autologous Lymphocytes Transduced with a Ribozyme that Cleaves HIV-1 RNA.*
RAC Approval: 9-10-93

9309-058 (T) Economou, James S. and Glasby, John A.; University of California Medical Center, Los Angeles, California; *Genetically Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma.*
RAC Approval: 9-10-93/NIH Approval: 12-2-93

9312-059 (T) Oldfield, Edward H. and Ram, Zvi; NIH, Bethesda, Maryland; *Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis.*
RAC Approval: 12-2-93/NIH Approval: 1/20/94

9312-060 (T) Sobol, Robert E. and Royston, Ivor; San Diego Regional Cancer Center, San Diego, California; *Injection of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2.*
RAC Approval: 12-2-93

9312-061 (T) Schuening, Friedrich; Fred Hutchinson Cancer Research Center, Seattle, Washington; *Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.*
RAC Approval: 12-2-93

9312-062 (T) Haubrich, Richard; University of California at San Diego Treatment Center, San Diego, California; *An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT(V) (HIV-1 IIIBenv/Retroviral Vector) in HIV-1 Infected Subjects.*
RAC Approval: 12-3-93

9312-063 (T) Sznol, Mario; NIH, Frederick, Maryland; *A Phase I Trial of B7-Transfected Lethally Irradiated Allogeneic Melanoma Cell Lines to Induce Cell Mediated Immunity Against Tumor-Associated Antigens Presented by HLA-A2 or HLA-A1 in Patients with Stage IV Melanoma.*
RAC Approval: 12-3-93

9312-064 (T) Rubin, Joseph, Mayo Clinic, Rochester, Minnesota; *Phase I Study of Immunotherapy of Advance Colorectal Carcinoma by Direct Gene Transfer into Hepatic Metastases.*
RAC Approval: 12-3-93

9312-065 (T) Chang, Alfred E., University of Michigan, Ann Arbor, Michigan; *Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene.*
RAC Approval: 12-3-93

9312-066 (T) Sorscher, Eric J. and Logan, James L.; University of Alabama, Birmingham, Alabama; *Gene Therapy for Cystic Fibrosis Using Cationic Liposome Mediated Gene Transfer: A Phase I Trial of Safety and Efficacy in the Nasal Airway.*
RAC Approval: 12-3-93

9312-067[a] (T) Welsh, Michael J.; Howard Hughes Medical Institute, Iowa City, Iowa; *Adenovirus-Mediated Gene Transfer of CFTR to the Nasal Epithelium and Maxillary Sinus of Patients with Cystic Fibrosis.*
RAC Approval: 12-3-93/NIH Approval: 2-10-94
Minor Modification: 2-17-94

	T	M	Total (T + M)
RAC APPROVED	44	23	67
NIH DIRECTOR APPROVED	35	23	59[c]

LEGEND:

M = Human Gene Marking Protocol
T = Human Gene Therapy Protocol
[a] = Minor Modification(s)
[b] = Major Amendment(s)
[c] = The number of protocols appearing in the table are greater than the number that appears in the protocol list because of the single *Expedited Review* protocol that was approved by the NIH Director on 12-28-92.
_ = RAC Approved/NIH Approval Pending

March 2, 1994

MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
3/30/90	3/2/89	8810-001	Rosenberg, Steven A.	The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2, and Tumor Infiltrating Lymphocytes	Lift the cap on the number of patients accrued onto the protocol.
11/91	7/12/91	9102-004	Brenner, Malcolm K., et. al.	Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-005	Brenner, Malcolm K., et. al.	A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-006	Brenner, Malcolm K., et. al.	A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement	Use the retrovirus vector, G1Na, instead of LNL6.
7/9/92	10/15/91	9110-010	Rosenberg, Steven A.	Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF)	Extend patient eligibility criteria to include patients with advanced breast cancer.
11/30/92	1/17/92	9105-009	Lotze, Michael T.	The Administration of Interleukin-2~~, Interleukin-4,~~ and Tumor Infiltrating Lymphocytes to Patients with Melanoma	(1) Eliminate systemic IL-4 therapy, and (2) allow administration of cyclophosphamide prior to TIL infusions.
12/3/92	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy of Familial Hypercholesterolemia	Increase patient accrual from 3 to 5 patients.
1/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	(1) addition of assays for anti-DNA antibodies on day 28 and week 8; (2) repeat chest and abdominal CTs, when indicated, at 6-8 weeks following initial treatment; (3) additional biopsies performed on days 16 and 20 and as needed to verify gene expression; (4) reduce the duration of the protocol from 16 to 8 weeks; and (5) expand patient eligibility to include those patients who have tolerated previous treatments without side effects at higher doses.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
1/22/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	Deliver HLA-B7 DNA liposome complexes by catheter to pulmonary nodules.
3/31/93	8/14/92	9206-022	Gansbacher, Bernd, et. al.	Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma	Use only irradiated SK-RC-39 cell line, not SK-RC-28.
4/19/93	7/12/91	9105-007	Deisseroth, Albert B.	Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial	New transduction protocol which allows the use of autologous stroma and growth factors.
4/19/93	8/14/92	9206-020	Deisseroth, Albert B.	Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy	Revised transduction procedure that includes the use of autologous stroma and growth factors.
4/21/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	Treat newborns with ADA deficiency with ADA gene-corrected autologous hematopoietic stem cells obtained from the patients' umbilical cord and placenta.
5/17/93	4/16/93	9212-034	Crystal, Ronald G.	A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient. Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.	Decrease the "baseline" and "vehicle control" portions of the protocol from 1 month to 1 week each.
6/7/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	The investigator is permitted to: (1) withdraw PEG-ADA at his discretion, and (2) perform bone marrow aspirations as necessary to monitor for gene transduction.
8/6/93	2/5/93	9202-016	Freeman, Scott M.	Gene Transfer for the Treatment of Cancer	PI has moved to Tulane University Medical Center. The total number of patients will be divided between Tulane University and the University of Rochester.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
8/18/93	8/25/93	9212-035	Wilson, James M.	Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/19/93	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy for Familial Hypercholesterolemia	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/26/93	8/26/93	9212-035	Wilson, James M.	Gene Therapy for Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial	(1) the lowest dose of adenovirus vector will be decreased by 100-fold, (2) the highest dose will be decreased by 10-fold, (3) the incremental dose between the patient groups will be reduced from 10-fold to 3-fold, (4) the number of patients within each group will be reduced from three to two, and (5) the total number of patients enrolled will be increased from 12 to 20.
10/8/93	4/16/93	9212-034	Crystal, Ronald, G.	Phase I Study, In Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung	(1) Change in dosing schedule, (2) increase time interval between nasal and lung administration from 1 to 2 days, (3) the time interval between patients within each dosing group will be changed to 2 weeks, (4) the time interval between the dose groups will be changed to 3 weeks, (5) isolation period will be reduced from 10 to 2 days, (6) eliminate requirement for negative pressure isolation of patients, (7) inpatient requirement will be reduced from 2 months to 2 weeks (contingent on 3 consecutive negative cultures), (8) addition of bronchial biopsies, (9) elimination of scintigraphic assessment, (10) elimination of quantitation of sputum volume and sputum culture, (11) patients will enter the 7 day vehicle control period within 2 months of completion of the 7 day baseline period and will receive vector administration within 2 months of completion of the vehicle control period, and (12) the number of chest CT scans will be reduced to 1 baseline scan unless indicated for clinical purposes. Contingency: any patients demonstrating unexpected side effects will receive a CT scan.

DATE OF MINOR MODIFICATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
10/18/93	4/16/93	9212-036	Welsh, Michael J.	Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium	(1) add an additional 3 patients; (2) each additional patient will receive $5x10^7$ IU of the adenovirus vector; (3) the adenovirus vector will be applied to only 1 nostril (saline in the other as a control); (4) the virus treated and saline control tissues will be biopsied (patient #1-day 1, patient #2-day 3, and patient #3-day 7); (5) use an alternative method of vector delivery (small catheter); (6) delete adenosine administration, (7) replace skin patch with subcutaneous needle for nasal epithelium voltage measurements; (8) decrease the period of hospitalization from 2-3 weeks to 5-7 days; and (9) eliminate nasal brushings.
11/3/93	12/2/93	9209-030	Deisseroth, Albert B.	Use of Retroviral Markers to Evaluate the Efficacy of Purging and to Discriminate Between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Neoplastic Cells in Autologous Marrow Following Purging in Patients with Chronic Lymphocytic Leukemia (CLL)	(1) Addition of PIXY321 growth factor during retrovirus transduction, and (2) perform stromal monolayers 2 weeks before retrovirus transduction.
11/18/93	12/2/93	9209-030	Deisseroth, Albert B.	Use of Retroviral Markers to Evaluate the Efficacy of Purging and to Discriminate Between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Neoplastic Cells in Autologous Marrow Following Purging in Patients with Chronic Lymphocytic Leukemia (CLL)	Expand patient eligibility criteria to include indolent non-Hodgkin's lymphoma (NHL) patients. The revised protocol title reads: Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients
11/29/93	4/16/93	9212-034	Crystal, Ronald G.	Phase I Study, in Cystic Fibrosis Patients, of Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung	(1) Expand the CFTR genotype eligibility criteria from the delta F508 genotype to "any CFTR genotype", and (2) delete the 3-day waiting period between the baseline and vehicle control periods and between the vehicle control and experimental treatment periods.
11/29/93	8/14/92	9206-019	Oldfield, Edward H.	Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir	The number of patients accrued onto the study will remain the same; however, the numerical constraints as to each diagnostic group (primary versus metastases) will be eliminated.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
1/6/94	8/14/92	9206-023	Dunbar, Cynthia	Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Myeloma	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
1/6/94	8/14/92	9206-024	Dunbar, Cynthia	Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Breast Cancer	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
1/6/94	8/14/92	9206-025	Dunbar, Cynthia	Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
2/4/94	4/16/93	9212-034	Crystal, Ronald G.	Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication-Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung	(1) The protocol may be conducted at additional sites (the University Hospital, Rockefeller University, New York, New York, and the New York Hospital-Cornell Medical Center, New York, New York); and (2) nasal administration of the AdCFTR vector, without lung administration, may be conducted on an additional 14 patients; and (3) the dosing schedule may be modified in accordance with FDA requirements.
2/17/94	9/6/90	9007-003	Rosenberg, Steven A.	Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor	The transmembrane portion of the tumor necrosis factor gene may be replace with the γ-IFN signal peptide.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
2/17/94	10/7/93	9303-042	Boucher, Richard C. Knowles, Michael R.	Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity	(1) Cystic fibrosis patients and healthcare workers will not be required to demonstrate neutralizing antibodies to adenovirus type 5 (Western blot and ELISA confirmation will be maintained), and (2) an additional 3 patients may be accrued onto the study at a lower starting dose of 10^7 pfu/ml.
2/17/94	2/10/94	9312-067	Welsh, Michael J.	Adenovirus-Mediated Gene Transfer of CFTR to the Nasal Epithelium and Maxillary Sinus of Patients with Cystic Fibrosis	A duplicate protocol may be initiated at the University of Washington, Seattle, Washington, in addition to the University of Iowa College of Medicine, Iowa City, Iowa. The PI will be responsible for quality control and semi-annual data reporting for research conducted at both locations.
2/25/94	12/2/93	9306-044	Deisseroth, Albert	Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial	(1) IL-3 and IL-6 may be added during the ex vivo transduction procedure, and (2) the eligibility criteria may be modified.
2/25/94	12/2/93	9306-044	Deisseroth, Albert	Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial	(1) Patient eligibility will be limited to patients with stage III/IV ovarian cancer; (2) patients who have received carboplatin (doses <300mg/m^2 <2 weeks prior), may be excluded; (3) patient accrual will be limited to 10 fully evaluable patients (max. of 20 patients entered); (4) exclusion of patients with >grade 1 neuropathies; and minor revisions to the protocol and Informed Consent document.
2/25/94	2/5/93	9209-027	Schuening, Friedrich	A Phase I/II Study of the Use of Recombinant Human Interleukin 3 Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation (ABMT) in Patients with Breast Carcinoma or Hodgkin's Disease	(1) Revised title is: A Phase I/II Study of PIXY321, a rhGM-CSF/rhIL-3 Fusion Protein, for the Mobilization of Peripheral Blood Stem Cells for Autologous Stem Cell Transplantation; (2) rhIL-3/GM-CSF (PIXY321) will be used to mobilize peripheral blood stem cells instead of rhIL-3; (3) peripheral blood stem cells alone may be used for transplantation instead of peripheral blood stem cells and additional bone marrow cells; (4) patient eligibility is modified to include non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, breast cancer, and other solid tumors; and (5) the cytoreduction chemotherapy regimen may be modified.

DATE OF MINOR MODIFICATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
2/25/94	2/5/93	9209-028	Schuening, Friedrich	Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies	This study will be merged with protocol # 9209-027; therefore, this protocol is considered closed.

ORDA/February 28, 1994

GLOSSARY

AAT	alpha-1-antitrypsin
AIDS	aquired immune deficiency syndrome
AZT	azidothymidine
cDNA	complementary deoxyribonucleic acid
CMV	cytomegalovirus
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
env	envelope
G-CSF	granulocyte colony stimulating factor
γ	gamma
GTAC	Gene Therapy Advisory Committee of the United Kingdom
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
IRB	Institutional Review Board
kb	kilobase
neo^R	neomycin resistance
NIH	National Institutes of Health
NIH Guidelines	*National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules*
ORDA	Office of Recombinant DNA Activities
PCR	polymerase chain reaction
Points to Consider	*Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects*
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
TIL	tumor infiltrating lymphocytes
TNF	tumor necrosis factor
ζ	zeta

HUMAN GENE TRANSFER PROTOCOL

9403-068 Rosenblatt, Joseph; University of California, Los Angeles, California; Seeger, Robert, Childrens Hospital, Los Angeles, California, and Merritt, James A.; Viagene, Inc., San Diego, California; *A Phase I Study of Immunization with Gamma Interferon Transduced Neuroblastoma Cells.*

Date of RAC Approval: 3-3-94
Date of NIH Approval: 10-25-94

SCIENTIFIC ABSTRACT

A Phase I/Ib trial using neuroblastoma cells genetically engineered to express gamma interferon (IFN-γ), using a retroviral vector is proposed, in an effort to achieve a therapeutic antitumor immune response. Neuroblastoma is the most common extracranial solid tumor of childhood. Sixty percent of neuroblastoma patients are at risk for developing fatal progressive disease. Despite the success of induction chemotherapy/myeloablative therapy and autologous transplant in attaining complete clinical remissions in the high risk group, approximately 60% develop progressive disease and die. Treatment with retroviral vector mediated transfer/expression of γ-IFN into neuroblastoma cells, is proposed as a means of evoking an active immune response. Autologous neuroblastoma cells will be engineered to express γ-IFN where available. If autologous neuroblastoma cells are not available, single HLA haplotype matched allogeneic cells will be used as an immunogen. Patients at high risk of relapse with minimal or no detectable disease following myeloablative therapy and autologous bone marrow transplant, or patients with progressive/persistent disease despite conventional therapy will be serially immunized with autologous/allogeneic neuroblastoma cells engineered to express γ-IFN. Tumor cell lines will be established from tumor tissue or bone marrow metastasis, transduced with γ-IFN vector, and transduced cells selected for G418. Levels of γ-IFN production, MHC-I and MHC-II expression will be characterized. Following selection, and characterization, transduced cells will be lethally irradiated prior to use as an immunogen. The study will characterize safety/toxicity, clinical, and biological (immune) response.

NON-TECHNICAL ABSTRACT

Neuroblastoma is the most common solid tumor (cancer) to occur outside of the brain in children. Although high doses of chemotherapy followed by bone marrow transplantation can achieve a complete remission in a large percentage of patients, most (60%) of high risk patients will subsequently relapse and die of progressive disease. We wish to use a novel gene therapy approach to try to eradicate the relatively small number of cancer cells that survive maximal chemotherapy, or to treat progressive disease in patients that have failed therapy. To accomplish this we will "insert" genetic material (DNA) designed to express human gamma interferon (γ-IFN), using a DNA delivery system known as a retroviral vector, into tumor cells obtained from the patient or cells from neuroblastoma patients with a shared tissue type (HLA type). We will then inject γ-interferon producing neuroblastoma cells (γ-IFN) in an effort to provoke an "immune" response against the cancer cells. The purpose of this study is to determine the maximal tolerated dose of such genetically engineered cells, and to obtain preliminary information regarding efficacy. Injection of γ-IFN producing tumor cells has resulted in protective immunity against "non-engineered" parental tumor cells in some animal models.

Tumor cell lines will be derived from clinical specimens, obtained at the time of surgical resection of tumor, from diagnostic bone marrow aspirations, or from peripheral blood. These tumor cell lines will be infected with a γ-IFN producing retroviral vector prepared by Viagene Inc. (San Diego). Following this, we will select for "engineered" tumor using the antibiotic G418 (which kills cells that do not carry the vector) and measure levels of γ-IFN production. We will test for sterility of the engineered cells, and freeze them for storage until needed. Just prior to use, they will be thawed, irradiated (with X-rays) to make sure they cannot grown in the patient. We will then inject various doses of tumor cells into the patient under the skin, according to a schedule outlined in the protocol, at 2 week intervals. We will determine whether this approach is safe, whether it increases the number of patients who remain disease free, whether it can decrease the size of tumor which is already present, and finally, whether a specific immune response can be measured in the laboratory following vaccination with genetically engineered neuroblastoma cells.

A PHASE I STUDY OF IMMUNIZATION WITH GAMMA INTERFERON TRANSDUCED NEUROBLASTOMA CELLS

STUDY COMMITTEE

Robert C. Seeger, MD	**Co-Chairman (CHLA)**
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Beth Frederick, R.N., MSN	Nursing (CHLA)
James Economou, MD, PhD	Gene Transduction (UCLA)
Stephen A Feig, M.D.	Oncologist (UCLA)
Randal K. Wada, MD	Oncologist (UCLA)
Katherine K. Matthay, MD	Oncologist (UCSF)
Jack Barber, PhD	Cancer Immunotherapy (Viagene)
Ram Gangavalli, PhD	Cancer Immunotherapy (Viagene)
Charles Prussak, PhD	Vector Manufacturing (Viagene)
David Hartnett	Vector Quality Control (Viagene)

PARTICIPATING INSTITUTIONS

Childrens Hospital Los Angeles
UCLA School of Medicine
UCSF School of Medicine

1.0 OBJECTIVES AND RATIONALE

Objectives: Treatment with retroviral vector-mediated gene transfer of the gamma interferon (γ IFN) gene into neuroblastoma cells is proposed as a means to improve active specific immunotherapy for human neuroblastoma. Autologous cells will be employed where available. In patients for which an autologous neuroblastoma cell line is not available, single haplotype matched allogeneic neuroblastoma cells will be used. The therapy is proposed for patients at high risk of relapse with minimal or no detectable disease following myeloablative therapy and autologous bone marrow transplant; and for patients with persistent/progressive disease following conventional therapy. The potential increase in specific immunity against immunogenic gene-modified neuroblastoma cells may result in an immunologic response resulting in eradication of residual microscopic disease, or a regression/retardation of progression of persistent tumors.

Our long term goal is to improve the outcome of patients with high risk neuroblastoma. Although effective induction chemotherapy followed by high dose myeloablative chemotherapy and autologous bone marrow transplant (ABMT) achieves complete remission in up to 80% of such patients, over 50% of these develop recurrent fatal disease (62,63,71). Biologically based therapy may be able to eliminate the relatively small number of residual tumor cells that survive maximal chemotherapy, surgery, and irradiation. The hypothesis of the project is that active specific immunotherapy with neuroblastoma cells engineered genetically to evoke an anti-tumor response may have an important role in eliminating minimal residual disease, and possibly a role in treatment of progressive disease as well.

To test the hypothesis we will introduce cytokine genes expressing gamma interferon into autologous neuroblastoma cells, and test the ability to elicit a specific response in a Phase I Protocol. As not all patients will have autologous tumor cells available, we will also investigate whether cytokine producing single HLA locus matched allogeneic neuroblastoma cells can serve as an immunogen. For these latter patients we will utilize a neuroblastoma cell bank which currently contains approximately 140 different human cell lines to develop a panel of cytokine transduced tumor cells. We have initiated HLA typing of these cells to identify cell lines with common (eg A2, A1) subtypes suitable for inclusion in this study.

Neuroblastoma, a tumor of the peripheral nervous system, is the most common extra-cranial solid tumor of childhood and has an incidence of 1 per 7000 children under the age of 15 (61). Approximately 60% of children with neuroblastoma are at high risk for developing fatal progressive disease despite all available current therapies (62,63). The group includes patients with stage IV disease diagnosed after 1 year of age, and patients whose tumors have amplification of the N-*myc* oncogene, regardless of stage of disease or age of diagnosis (63-65). Ability to derive an immortalized cell line is itself a strong predictor of high risk (Fig. 12). Current data suggests that high risk patients should receive aggressive therapy followed by high dose consolidation therapy (63). With myeloablative therapy and ABMT, nearly all patients achieve a complete clinical remission, but the tumor recurs in over 60% of patients indicating the need for better therapy (62,63,71). Patients who relapse following induction /ABMT generally have survival measuring 4 months or less. Relapses in high risk patients generally occur within 2 years of completion of therapy. Our investigations are designed to develop gene transfer based therapy that can effectively eliminate tumor cells surviving maximal chemotherapy, and which may alter the course of patients who have progressive disease despite failure of available therapies.

Most neuroblastoma cells have reduced expression of Class I and Class II major histocompatibility complex (MHC) antigens (1,65,70,75). A potential strategy in immunotherapy involves presentation of tumor antigens in the context of MHC class I and class II (2,20,22) (Figure 1). Immunogenicity of tumor cells may be augmented in an antigen specific manner by stimulation of MHC molecules. γ-IFN is known to induce both class I and class II MHC expression (13,15,17,18,28,30,76,78). We have transduced multiple human neuroblastoma cell lines with a retroviral based human γ-IFN vector (DAHu γ-IFN). Our preliminary results demonstrate that γ-IFN vector can reliably transduce up to 2/3 of available neuroblastoma cell lines (Table IV) and that transduced cells produce high levels of γ-IFN, and markedly upregulate MHC class I and class II (Table IV, V, Figures 14,15) as well as some neuroblastoma associated antigens (NAAg) (Figure17).

Anti-tumor response has been enhanced in vitro and in people by expressing a large variety of cloned cytokines in tumor cells (22,24-30,74). Anti-tumor effects have been observed with transduction of γ-IFN into mouse fibrosarcoma, renal cancer, and neuroblastoma cells (28-30). Several cytokines have been shown to augment the immune response to neuroblastoma in animal models (28,72). γ-IFN secretion by tumor cells is known to decrease

tumorigenicity and has induced persistent anti-tumor immunity in several animal models. In a renal cell cancer model, extent of modulation of MHC class I and class II correlated with response to interferon gene therapy (30).

In contrast to many other solid tumors, neuroblastoma cells grow readily in vitro (64,65). Cell lines are readily derived both from biopsy tissues as well as from bone marrow and occasionally peripheral blood. Ability of the human neuroblastoma to form a cell line is associated with poor prognosis (Figure 12). Thus neuroblastoma is an excellent candidate for gene therapy. The advantages of neuroblastoma as a model system include ability to grow cells in vitro, ability to achieve complete remission with standard therapy, and the high and predictable relapse rate. Hence we wish to test modern gene therapy strategies for neuroblastoma using a retroviral vector designed to express γ-IFN. The study is designed to address safety, clinical response (disease free interval, relapse rate, tumor burden) and biologic response (immune response).

2.0 INTRODUCTION

2.1 CANCER IMMUNOLOGY

Host immunity is an important mechanism by which malignant tumors can be kept in check or even cleared. Established tumors are therefore under selective pressure to develop means of evading the immune system. Although the mechanisms by which tumors evade the immune system are undoubtedly varied and complex, at least one evasion mechanism appears to derive from the fact that the levels of Class I MHC proteins are unusually low on some tumors (1, 2). Since Class I MHC proteins are essential for the presentation of intracellular antigen to the immune system (3,4,66), suboptimal levels of MHC proteins may prevent effective presentation of tumor-specific antigens. The immune system is therefore relatively blind to the tumor cell, at least with regard to MHC Class I-restricted cytotoxic T-lymphocytes (CTL). A second mechanism by which tumors could evade the immune system is by developing anergy due to generation of suppressor T cells or other immune depressing substances to prevent the normal activation of anti-tumor immunity (immune "anergy")(5-7). This may include a block to MHC-II restricted help needed for generation of CTL. Effective immunotherapy strategy must necessarily overcome one or both of these phenomena.

Methods for improving the immune responses against tumors, especially CTL responses, are likely to be therapeutically beneficial to cancer patients. A variety of approaches have been used to engender non-specific augmentation of the immune systems of cancer patients. For instance, lymphokines such as IL-2, α-IFN, and more recently, γ-IFN have proven to be therapeutically useful, although efficacy is limited and toxicity associated with systemic administration is considerable (8-10, 73). γ-IFN and IL-2 presumably function as growth and activation factors (11-16) to tumor-reactive immune cells such as CTL, macrophages, natural killer (NK), and lymphokine activated killer / tumor infiltrating lymphocytes (LAK/TIL) at the site of the tumor, possibly to overcome immune suppression. γ-IFN also increases the expression of MHC proteins on the surface of tumor cells to improve antigenic presentation (17,18,28,75,76). However, systemic administration of lymphokine may only marginally contribute to tumor rejection, and is often limited by significant toxicity. Systemic injection of lymphokines is thus a highly inefficient way to deliver these critically important proteins to their desired site of action, the tumor.

One approach that would assure the continued presence of γ-IFN locally around tumor cells is to introduce constitutively expressed lymphokine genes into tumor cells. The resultant constitutive, low level production of γ-IFN should specifically increase the level of MHC (the antigen presenting proteins) on the surface of tumor cells. In addition, γ-IFN should be secreted locally around the tumor and thus aid the growth and activation of local immune cells (such as CTL, macrophages, and NK cells, as they respond specifically to tumor antigen. The concentrated, local presence of the immune-activating γ-IFN may thus help overcome immune anergy. The total amount of lymphokine should then be quite low, but it should be concentrated where it would be most effective, namely at the site of tumor cells. The overall effect of lymphokine gene transfer would be to "flag" the tumor cell as something that the immune system should carefully scrutinize and respond against. The resultant boost in tumor specific immunity could result in improved responses compared with non-gene-modified approaches (Fig 1).

We (see section 3.2) and others (24-30) have observed the potential utility of retrovirally mediated lymphokine gene delivery in general, and γ-IFN gene delivery specifically (28-30), in murine tumor model systems. The data have been sufficiently encouraging to warrant clinical investigation in cancer patients. We therefore propose to investigate the utility of this approach in human neuroblastoma.

2.2 RETROVIRAL VECTOR PACKAGING CELL LINES

Retroviruses are positive-strand RNA viruses that pass through a double-stranded DNA stage after infection (31,32). Viral DNA integrates into the host genome to form a provirus, which contains the viral genes encompassed on either end by viral long terminal repeat (LTR) elements which are required for integration. After integration, viral genes are expressed from the provirus by utilizing host cell machinery and transcriptional promoter and termination *cis* elements found within the viral LTRs. The full length viral RNA transcript (the next generation viral genome) is recognized via a specific "packaging signal" (Ψ) found in the viral RNA genome and encapsidated into an infectious viral particle by the viral proteins. The retroviral life cycle is generally non-lytic for the cell, and allows continuous viral production over a long period of time.

The retroviral vector used in these studies is based upon Moloney murine leukemia virus (MoMLV), that was engineered so that its extraneous viral coding sequences have been replaced with a desired gene (32-34), such as γ-IFN. Unlike true viruses, the recombinant vector is incapable of replication since its genome lacks all of the viral structural genes. Any gene of interest can replace the protein coding region of the viral genome and become packaged within a packaging cell line (PCL) into virion particles for transduction, provided that it is contiguous with the essential *cis* elements (i.e., Ψ, LTRs, and sequences required for priming of DNA synthesis) of the genome.

The viral proteins needed for encapsidation and vector production must then be supplied in a PCL. The PCL is a cell line which expresses the MoMLV structural proteins, and as a result produces empty virion particles which lack the viral genome required for replication. Viral provector DNA introduced into the PCL will act as a template for the production of the RNA vector genome, which is then packaged into the previously empty virions, generating retroviral vector particles (33,34).

For clinical studies, the most important safety concern with the use of retroviral vectors is the inherent propensity of murine retroviral PCLs to generate replication-competent retrovirus (RCRV) after introduction of a vector (33-36). This can theoretically occur by homologous recombination between the retroviral provector DNA and either 1) the DNA encoding the MoMLV structural genes present in the PCL (36), or 2) the endogenous proviruses found universally in murine cells (37), the species from which all the MLV-based PCLs previously used for human gene therapy studies were generated. In fact, even murine cell lines lacking vector can be induced to produce xenotropic RCRV (38) which can replicate in human cells.

Another safety concern with murine PCLs used for retroviral vector production is that endogenous defective proviruses in the murine genome are expressed (e.g., VL30 sequences), recognized by the retroviral structural gene products of murine PCLs, and delivered and expressed in target cells with an efficiency at least comparable to that of the desired vector (39). The delivery of these undesirable retroviral-like sequences and the possibilities for generating replication-competent retrovirus suggest that murine cell lines may be less than ideal for the production of murine retroviral vectors for human therapeutics.

Due to the safety risks associated with the generation of RCRV by homologous recombination with endogenous sequences, we have developed MLV-based retroviral PCLs from a canine cell line, D17. The MLV structural genes ("gag/pol" and "env") are also genetically unlinked, to decrease the chance of retrovirus generated by homologous recombinations between the therapeutic pro-vector DNA and the DNA encoding the MoMLV structural genes present in the PCL. These changes should help alleviate many of the safety concerns described above. These vectors have been previously used in several RAC approved gene therapy protocols.

2.3 CLINICAL APPROACH

We propose to utilize retroviral vectors to deliver and express the γ-IFN gene to human tumors in order to improve active immunity for human malignancies. It is hoped that this approach will improve antigenic presentation and subsequent tumor-specific immune activation in cancer patients by creating an optimal microenvironment around the tumor cells for the activation of cell mediated immune responses. Short-term tumor cell cultures will be established from human tumor biopsies and/or bone marrow metastases. The resultant cell cultures will be transduced with the γ-IFN retroviral vector. After G418 selection, lethal irradiation, and quality control testing, the lymphokine-expressing autologous tumor cells will be injected back into the patient. An increase in specific immunity against the gene-modified tumor cells may result in the clearance of the endogenous unmodified metastatic tumors (see Fig 1 for a diagrammatic summary of the approach).

Neuroblastoma is the most common extracranial solid tumor of childhood, and has an annual incidence of one per 7000 children under age 15. Neuroblastomas differ markedly in their clinical behavior. Sixty percent of patients are at high-risk for developing progressive and ultimately fatal disease. In high-risk patients 5 year survival rates are low (40%) even with use of intensive chemotherapy followed by BMT (62,68,71). Therefore, additional therapeutic strategies are required to improve the outcome in high-risk neuroblastoma.

In contrast to many other solid tumors, neuroblastoma cells grow readily in vitro. In fact, the ability of a human neuroblastoma to form a cell line is strongly associated with a poor prognosis (Fig.12). Thus, neuroblastoma is an excellent candidate for gene therapy. The advantages of neuroblastoma include: i) ability to grow cells in vitro, ii) ability to achieve minimal residual disease with standard therapy, and iii) the high and rapid relapse rate.

One commonly used strategy in tumor immunotherapy involve the induction of tumor antigens in the context of MHC. Antigen loaded MHC molecules are the preferred target antigens for T-cell mediated reactions. They serve to focus T lymphoctyes on those host cells that have foreign antigen on their surface. Class I MHC molecules (HLA A, B, C) are expressed on all nucleated cells and are recognized by cytotoxic T cells, whereas class II molecules (DP, DQ, DR) are typically confined to cells involved in immune responses (B cells, antigen presenting cells, thymus epithelial cells) and are recognized by helper T-cells. Immunogenicity of tumor cells may be potentially augmented in an antigen-specific manner by stimulation of the MHC class I molecules. Costimulation of T-cells by the CD28/CTLA4 ligand(s), B7/BB1, B7-2, may provide the antigen-nonspecific signal required for an immune response (68,69). γ-IFN has also been shown to augment B7 expression on some antigen presenting cells (Nadler L, personal communication).

Most neuroblastoma cells have reduced expression of class I and II MHC antigens (66,70,75). Therefore, if neuroblastoma cells can be genetically engineered to synthesize and secrete specific cytokines, effective tumor immunity may be induced by local secretion of these factors. γ-IFN has been found to have stimulating effects on expression of MHC class-I and II antigens on neuroblastoma (28,75). Most class I MHC genes have promoter sequences which are responsive to interferon (76). Antitumor effects have been observed with transduction of γ-IFN into mouse fibrosarcoma, and neuroblastoma cells (28,30): γ-IFN secretion by tumor cells decreased their tumorigenicity and induced persistent antitumor immunity. Furthermore, administration of γ-IFN transduced tumor cells into tumor bearing animals also resulted in substantial reduction of metastatic disease (82).

We will use a MoMLV-based retroviral γ-IFN vector to transduce neuroblastoma cell lines. We will then measure the expression of cytokine genes, alterations in MHC-I and II antigen expression, NAAg's, immunogenicity, and doubling time. The goal of these studies is to use retroviral vectors to render neuroblastoma cells immunogenic so that the transduced cells can be used as tumor vaccines, and to develop in vitro correlates of immunogenicity in neuroblastoma.

3.0 PRE-CLINICAL STUDIES

3.1 CHARACTERIZATION OF THE HUMAN γ-IFN VECTOR PRODUCING CELL LINE, VCLHUγ.

The γ-IFN proteins are highly species-specific and therefore the human γ-IFN gene must be used for human clinical studies. The structure of the provector is illustrated in Fig.2 (Appendix). The clonal vector producing cell line, VCLHuγ, has been carefully characterized with regard to the possible presence of adventitious agents (summarized in Table I). The cell-free supernatant results in an average titer of 3.7×10^6 cfu/ml and several tests have failed to indicate the presence of RCRV. Co-cultivation of the VCLHuγ vector producing cell line with Mus dunni cells (to amplify retrovirus, if present) also fails to detect RCRV. The cell line lacks adventitious agents as determined by broth and agar culture assays (for bacteria, fungi, and mycoplasma) as well as Hoechst staining (for mycoplasma). The cell line appears to have 1 provector with the appropriate genetic structure (Fig 3). Vector from VCLHuγ results in expression of biologically active γ-IFN in transduced cells (Table V) with the appropriate genetic structure (Fig.3). The VCLHuγ master cell bank has been characterized in accordance with the FDA "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1987)". These and other tests are summarized in Table I. The high titer and lack of adventitious agents make VCLHuγ ideal for the production of γ-IFN vector for clinical trials. This vector producing cell line is described in a Master File (BB-MF5110) submitted to the FDA and has already been approved by the RAC for use in human melanoma, in a trial under the direction of Dr. Hilliard Seigler (RAC #9306-043).

3.2 Animal Model Studies.

The feasibility of the overall approach has been tested in several murine tumor model systems using retroviral vectors that express murine γ-IFN. Gene-modified murine tumor cell lines secreted γ-IFN and exhibited substantial increases in the surface expression of Class I MHC proteins (28-30). The modified tumor cells were substantially less tumorigenic, apparently due to their ability to invoke increased anti-tumor immunity. Further, injection of γ-IFN-expressing tumor cells resulted in potent CTL activity capable of specifically lysing the unmodified parent tumor cell (29). Unmodified tumor cells did not engender such a response. Pre-injection with irradiated γ-IFN-expressing tumor cells, but not the unmodified tumor cells, protected animals from subsequent challenge with the unmodified tumor and has also "cured" metastatic disease in tumor bearing animals (29). This effect is presumably due to the observed induction of the tumor specific CTL. These data show that the naturally poor immunogenic properties of tumor cells can be improved by the constitutive expression of γ-IFN delivered by retroviral vectors. The immune system, thus primed, can mount potent responses against the unmodified parent tumor. This conclusion suggests that a similar gene transfer based immunotherapeutic approach to human cancer may substantially benefit cancer patients.

3.2.2. MURINE γ-IFN VECTOR EXPRESSION AND MHC. The retroviral vector containing the murine γ-IFN gene was generated and introduced into several murine tumor cell lines; L33 (fibrosarcoma), B16F10 (melanoma), CT26 (colo-rectal carcinoma), and Lewis lung carcinoma. All transduced lines produced measurable levels of biologically active γ-IFN detectable in the growth media compared with the non-transduced parent tumor cell lines (Table IV). Transduction by murine γ-IFN vector substantially increased the level of Class I MHC (Fig.4). Since these proteins present antigen to the immune system, the effect of vector transduction may be to improve immune responses, especially those that are Class I restricted such as CTL.

3.2.3. TUMORIGENICITY OF MURINE γ-IFN-EXPRESSING CELLS. Improved immune responses against γ-IFN-transduced tumor cells might be reflected in a decrease in the tumorigenic properties of the tumor. The effect of endogenous γ-IFN expression on the tumorigenic properties of the vector modified tumor cells was therefore assessed in syngeneic animals. Vector-modified B16F10, CT26, and Lewis lung were all substantially less tumorigenic than the corresponding unmodified parent tumor cell lines. The data in Fig.6 indicate that γ-IFN transduced L33 fibrosarcoma cells are non-tumorigenic in immune competent animals.

In addition, recombinant γ-IFN protein added exogenously to the L33 cells did not affect tumorigenicity (Fig 6), even though control experiments indicate that treatment resulted in high level MHC expression for as long as 3 days after treatment (data not shown). This indicates that the effect of constitutive expression of γ-IFN obtained by gene transfer can be superior to the effect obtained by treatment with recombinant γ-IFN protein. Control experiments with vectors which express only Neo^r did not affect tumorigenicity (data not shown), indicating that the effect is due to γ-IFN expression per se and not due to the vector transduction process in general. This suggests that effects are due to local secretion of γ-IFN, rather than changes in antigenic phenotype due to exposure of cells to γ-IFN.

The tumorigenic properties of L33 γ-IFN-expressing tumor cell lines were also tested in immunocompromised athymic nude mice. The L33 tumors grew almost as well as the unmodified tumor (Fig 7), indicating that the decreased tumorigenicity observed in normal animals was at least partly due to an immune response involving T cells, most likely CTL.

3.2.4. GENERATION OF TUMOR-SPECIFIC CTL. The immunological mechanisms for the decreased tumorigenicity of tumor cells expressing γ-IFN were of interest. There were at least three possible explanations for immune protection observed for γ-IFN-expressing, but not unmodified, tumor cells. Either 1) the unmodified cells were not capable of eliciting cytotoxic lymphocyte responses, or 2) tumor-specific cytotoxic lymphocytes exist but the cells were resistant to lysis, or 3) both. The exceptionally low levels of Class I MHC on most tumor cells and the need for Class I MHC for antigen presentation (3, 4) suggested that immune responses against the γ-IFN-expressing tumor cells might not have been effective against the unmodified tumor.

To test these possibilities, the CTL induction characteristics of unmodified and γ-IFN-expressing tumor cells were compared for B16, Lewis lung tumor, and CT26, using the unmodified tumor cell as a target. Cytolytic activity was substantially increased for γ-IFN transduced B16 and Lewis lung tumor (data not shown). Representative data in Fig 8 indicate that injection of unmodified CT26 irradiated tumor cells resulted in a relatively poor CTL response. Injection with γ-IFN-expressing CT26, however, resulted in improved CTL responses. Control experiments using unrelated cell lines, BC10ME and B16F10 as CTL targets, indicated that the anti-CT26 immune response was specific (Fig 9). Antibody depletion studies indicate that the CTL are CD8+ T cells (Fig. 10).

These data show that the lack of measurable response against the unmodified tumor is due to insufficient CTL induction, not due to the inability of the cell to be lysed by CTL. These results have been confirmed by other investigators with another tumor model (30, 82).

3.2.5. MURINE TUMOR VACCINATION STUDIES. To determine whether the observed immune responses could protect mice from challenge with unmodified tumor cells, vaccination studies were performed by priming mice with either unmodified, or γ-IFN-transduced, irradiated B16F10 tumor cells. The animals were then challenged with the unmodified parental tumor cell. A single pre-injection with as little as $4x10^5$ γ-IFN-expressing tumor cells, but not unmodified tumor cells, resulted in a measurable (~ 2.5-fold) protection against tumor challenge. A single injection with larger numbers of cells resulted a 5-fold decrease in the number of lung metastasis (Fig.11). γ-IFN-transduced tumor cells may therefore be capable of generating systemic, protective, anti-tumor immunity.

3.3. Human Neuroblastoma Data

3.3.1. MHC-I EXPRESSION IN NEUROBLASTOMA CELLS: In preparation for the current trial, we have characterized over 46 untreated neuroblastoma cell lines for overall expression of class I MHC using the W6/32 antibody. Only 5 of 46 tumors expressed class I antigens as determined by flow cytometry with W6/32 and most of these did so weakly (Reynolds CP, Seeger R, unpublished data; (64). Virtually all neuroblastoma subsets do not express MHC-I. Effective gene therapy strategy therefore would likely require induction of MHC-I.

Over 140 neuroblastoma cell lines have been established in the laboratories of Drs. Seeger and Reynolds, from tumor, bone marrow and/or blood of patients with neuroblastoma. As many as 70 of these lines have been recently established from patients who are still alive. Hence, a large variety of autologous and/or HLA partially matched allogeneic cell lines are available for the study. Our preliminary experience suggests that approximately two thirds of the lines will have adequate transduction growth characteristics, following γ-IFN transduction and will be available for use in the trial. However, a proportion of patients' cell lines do not grow adequately following γ-IFN transduction or may be difficult to transduce. The large neuroblastoma cell bank insures that for many of these patients, particularly those with common HLA haplotypes (eg A1, A2, etc.), suitable allogeneic cell lines will be available. We aim to develop a panel of transduced allogeneic cell lines for this purpose.

A variety of histologic/molecular characteristics portend a poor prognosis in neuroblastoma. These include aggressive histology, high mitotic index and N-myc amplification. However, one of the single worst prognostic determinants is the ability to establish a cell line in vitro. Hence, the availability of a cell line is predictive of a poor outcome (Figure 12). Therefore we are likely to indirectly select for patients most likely to benefit from any effective adjuvant therapy.

Initially we will try to identify HLA A2 haplotype cell lines, as this may be the most common haplotype. Since some neuroblastoma cells are known to express MAGE-1, the recently identified melanoma associated antigen recognized by cytolytic T-cells in patients with HLA-A1 haplotype (79), we will also try to identify cell lines from patients with A1 haplotype, particularly those that are MAGE-I expressors. Using molecular techniques, we have typed two of seven cell lines (LAN-1 and CHLA-127) as HLA-A2 haplotype, in collaboration with Dr. P. Terasaki (UCLA). These are presently undergoing γ-IFN transduction.

3.3.2 TRANSDUCTION OF NEUROBLASTOMA CELL LINES: A number of neuroblastoma cell lines have been transduced recently using the retroviral vector DA Huγ-IFN (Viagene, San Diego) or an alternative construct using the same vector backbone, but with γ-IFN sequences replaced by β-galactosidase coding sequences to assay for transduction efficiency. These are indicated below:

Cell line	Phenotype
LAN-1 :	N-myc amplified, MHC-I negative, A2 haplotype.
LAN-5:	N-myc amplified, MHC-I negative
LAN-6:	N-myc overexpressing, not amplified, weakly positive for MHC-I.
SK-N-AS:	N-myc non amplified, non expressing, MHC-I strongly positive.
SK-N-RA:	N-myc non amplified, MHC-I strongly positive
CHLA-125	Living patient, N-myc status not yet characterized, MHC-I not yet characterized.
CHLA-127:	Living patient, N-myc status unknown, MHC-I not yet characterized, A2 haplotype.

Conditions for G418 selection have been optimized using the above cell lines. G418 levels of 100 - 2000 μ/ml were tested for multiple lines. For most neuroblastoma lines, 500 μg/ml of G418 results in acceptable levels of cell death within 10-12 days. Optimal concentration and types of polycations for transduction have also been determined (Fig.13).

Levels of β galactosidase expression have ranged from less than 1% for LAN-6 to 12% for SKNRA without G418 selection (Table V). However, stable γ-IFN, neo^R transduced lines were readily derived from both cell lines. Levels of γ-IFN production for LAN-6, SK-N-AS and SK-N-RA are presented in Table V. Levels in transduced neuroblastoma cells have ranged from $12U/10^6$/day to as high as $596U/10^6$/day.

γ-IFN may produce some slowing of growth of some neuroblastoma cell lines. In addition, we have observed at least one cell line LAN-5, which was readily transduced using the β-gal vector (20% transduction efficiency at an MOI of 2) but for which stably transduced γ-IFN expressing lines could not be derived. This may be representative of the subset of patients for which allogeneic rather than autologous cell lines may need to be used, due to difficulties in outgrowth of γ-IFN producing cells, in some instances.

3.3.3. INDUCTION OF MHC CLASS I AND CLASS II EXPRESSION ON NEUROBLASTOMA CELL LINES: As had been hoped, γ-IFN transduction produced a variety of phenotypic changes in neuroblastoma cells, which may enhance immunogenicity and/or antigen presentation. Induction of MHC-I and class II expression was observed in all cell lines following transduction (Fig. 14, 15). Although similar MHC-I/II induction is observed with in vitro incubation with exogenous γ-IFN, rapid decline in MHC-I/II is observed following withdrawal of γ-IFN (Fig.16)..

As opposed to incubation with exogenous γ-IFN, MHC-I/II induction persisted over a one-two month period (Table V). Representative MHC-I induction in SK-N-AS, SK-N-RA and LAN-6 cells is shown in Table VI and Figure 14. Similar high grade induction of MHC-II was observed in SK-N-AS and SK-N-RA γ-IFN transduced cells (Figure 15). Levels of induction of MHC-I are comparable to those observed during incubation with exogenous γ-IFN at 100-1000U/ml. In addition to persistent MHC-I/II expression, the added effects of local γ-IFN production with respect to immune activation are not known. In contrast to MHC-I/II, we did not observe an increase in expression of the costimulatory ligand B7/BB1 as determined by flow cytometry in transduced SK-N-AS, and SK-NRA cells (data not shown). Whether any of the more recently

identified T-cell costimulatory ligands (eg B7-2 etc.) are induced is not known. However, γ-IFN is known to induce costimulatory CD28 ligand expression on some antigen producing cells (Nadler L, personal communication). γ-IFN transduction appeared to augment an allogeneic mixed lymphocyte-tumor cell reaction (MLTR) using transduced and nontransduced SK-N-RA cells, suggesting augmentation of antigen-presentation following transduction (Table VII). Whether the observed enhancement of MLTR is due to direct cytokine effects, phenotypic effects on tumor cells, or both, is under investigation.

In contrast to MHC-I/II, expression of most "tumor associated" antigens (NAAg), as determined by flow cytometry using monoclonal antibodies, is stable or actually enhanced. One such neuroblastoma antigenic determinant, HSAN1.2, was expressed at a higher level following transduction of SK-N-AS cells (Figure 17). Other neuroblastoma associated antigens (eg G_{D2}, 459, 390, and HNK-1) remained unchanged in expression. Whether antigen processing was altered in transduced cells is not known. However, continued expression of 5/5 NAAg suggests that γ-IFN will not substantially change tumor surface characteristics, and hopefully will augment presentation of NAAg, in the context of MHC-I/II. Whether any of the NAAg may serve as anitgenic targets is not known.

3.4 CONCLUDING REMARKS

The biological effects of γ-IFN transduced cells in vivo have been investigated using 4 distinct murine tumor models (a fibrosarcoma, a melanoma, a colorectal carcinoma, and a lung carcinoma). In all tumor models tested, we have shown that γ-IFN-transduced cells secrete biologically active γ-IFN, increase the level of surface-associated Class I MHC, and are non-tumorigenic due, at least in part, due to T cell responses, presumably CTL (see Table IV and Figs 5,6,7). CTL induction was stimulated with γ-IFN transduced tumor cells in all 3 tumor models tested (representative data is shown for CT26 in Fig.8). The CTLs thus generated were CD8+ and MHC-restricted (Figs.10). CTL generation due to injection of γ-IFN tranduced tumor cells resulted in systemic immunity to subsequent challenge with unmodified tumor in the B16 tumor model (Fig.11). Many of these observations have been substantiated by other investigators including Watanabe et. al. (28), Gansbacher et. al. (30), and Porgador et. al. (82). The latter investigators demonstrated that tumor-bearing animals could be cured of metastatic disease by subsequent injection of γ-IFN transduced tumor cells, but not by injection of unmodified tumor cells (82). Crowley and Seigler have also shown that injection of human anti melanoma CTL resulted in cleaning of human tumor xenografts in nude mice (80,81). Reproduction of these key references may be found in Appendix K.

In addition to these murine studies, we have also demonstrated the following: (1) human neuroblastoma cell cultures can be readily established and transduced with retroviral vectors expressing γ-IFN ; (2) the transduced neuroblastoma cells secreted γ-IFN and increased the expression of Class I and Class II MHC; (3) γ-IFN vector transduction of neuroblastoma cells resulted in potential improvement in antigen presentation as determined by in vitro stimulation of lymphocyte responses against allogeneic antigens. In addition, Seigler and coworkers (RAC protocol #9306-043) have shown that in vitro stimulation of patient PBMC with tumor cells transduced with the γ-IFN gene result in increased CTL activity against the unmodified tumor cell line (Fig. 18).

The use of γ-IFN transduced tumor cells has already been approved by the RAC for the treatment of melanoma patients (Seigler et. al., RAC protocol #9306-043). The parallel results observed in this proposal between in vivo murine studies and in vitro human neuroblastoma studies support the potential viability of the approach in neuroblastoma and warrant testing in high risk or relapsed neuroblastoma patients for which satisfactory alternative therapy is not currently available.

4.0 CLINICAL PROTOCOL (IRB# CCI# 93-103)

A PHASE I STUDY OF IMMUNIZATION WITH GAMMA INTERFERON TRANSDUCED NEUROBLASTOMA CELLS

1.0 GOALS AND OBJECTIVES

Active specific immunotherapy with neuroblastoma cells that have been genetically engineered to express cytokines may evoke an effective anti-tumor immune response by improving the microenvironment in which immune system cells can recognize and respond to tumor associated antigens. Neuroblastoma cells frequently do not express class I or II MHC molecules and thus are likely to be poorly immunogenic. We will transfect amphotrophic retroviral vectors which express interferon-γ (IFN-γ) cDNA into autologous or partially HLA matched allogeneic neuroblastoma cells to render them IFN-γ producers. IFN-γ has been shown to enhance expression of major histocompatibility complex (MHC) molecules on cell surfaces, which can result in their increased presentation of antigenic peptides to T lymphocytes. If IFN-γ transduction increases expression of MHC, tumor associated peptides may be more effectively presented by neuroblastoma cells to the immune system. In addition, a microenvironment of IFN-γ may facilitate processing and presentation of tumor associated antigens by macrophages to T lymphocytes.

High risk neuroblastoma patients will be studied in this phase I/Ib toxicity/immunogenicity trial. They include 1) those who have undergone myeloablative therapy and autologous bone marrow transplantation (ABMT) who have no detectible disease but who are at significant risk for recurrence; and 2) those with persistent or recurrent disease. Our aims are as follows:

1.1 To determine the maximum tolerable dose of IFN-γ transduced autologous or allogeneic human neuroblastoma cells when given as a subcutaneous injection.
1.2 To determine the local, regional, and systemic toxicities of IFN-γ transduced neuroblastoma cells.
1.3 To determine if IFN-γ producing tumor cells elicit an anti-neuroblastoma response *in vivo* as measured by standard clinical tests and immunocytologic evaluation of marrow metastases.

2.0 BACKGROUND

Neuroblastoma, which is a tumor of the peripheral nervous system, is the most common extracranial solid tumor of childhood and has an incidence of one per 7,000 children under the age of 15 years.[1] Neuroblastomas differ markedly in their clinical behavior, with low, intermediate, and high risk groups of patients being defined at diagnosis based upon expected outcome following conventional therapy.[2] Forty percent of children with neuroblastoma have low or intermediate risk tumors, and they nearly all become long-term, disease-free survivors following conventional therapy which includes surgery, local irradiation, and chemotherapy. However, 60% are at high risk for developing fatal progressive disease [3-8]. Our long-term goal is to improve the outcome of patients with high risk neuroblastoma. Although effective induction chemotherapy followed by high-dose, myeloablative chemoradiotherapy and ABMT achieves a complete remission in approximately 80% of high risk patients, 50% of these develop recurrent disease. Biologically based therapy, such as in this protocol, may be able to eliminate the relatively small number of tumor cells that survive maximal chemotherapy, surgery, and irradiation.

2.1 Preclinical Studies. Expression of MHC class-I molecules appears to be a critical determinant in rejection of tumor cells, since target cells must process and present peptide antigens in the context of class I molecules in order to be recognized and killed by CD8+ T lymphocytes.[9]

IFN-γ can increase the expression of MHC molecules in a variety of cell types. Antitumor effects have been observed after immunization of mice with IFN-γ transduced fibrosarcoma cells. IFN-γ secretion by CMS5 tumor cells decreased their tumorigenicity and induced antitumor immunity.[10] This appeared to be due to T cell immunity as judged by rejection of CMS5 tumor challenge and persistence of specific cytotoxic activity in the spleens of inoculated mice. Expression of IFN-γ cDNA in the C1300 mouse neuroblastoma cell line both enhanced MHC class-I expression and augmented antitumor immunity in the recipient.[11,12] The nonimmunogenic, low MHC class I-expressing methylcholanthrene (MCA) induced sarcoma cell line, MCA 101, is a poor presenter of endogenously generated viral antigens to CD8+ T lymphocytes and cannot be used to generate tumor infiltrating lymphocytes.

However, these cells, when transduced with IFN-γ generated a CD8+ T cell immune response that was against wild-type tumor cells.[13] The relationship between MHC class-I expression and tumor rejection may be complex, as higher levels of IFN-γ secretion are required in murine adenocarcinoma cells to elicit tumor rejection than are required to induce MHC class-I antigens.[14] Therefore, induction of tumor specific immunity by transduction of IFN-γ may include enhanced MHC class-I expression and other variables. The fact that these experiments have been successful in poorly immunogenic, syngeneic tumors of nonviral origin suggests that this strategy may be successful for human tumors of relatively low immunogenicity.

Tumor infiltrating lymphocytes (TIL) derived from patients whose melanoma cell lines had normal expression of HLA-A2 antigens were CD8+ and were capable of lysing autologous melanoma cells.[15] In contrast, TIL derived from patients whose melanoma cell lines did not express HLA-A2 were predominantly CD4+ and were not cytotoxic. These studies support the notion that enhanced MHC class-I expression could result in greater specific immunologic responses to tumors. Transduction of melanoma cells with IFN-γ enhanced expression of HLA molecules and their ability to stimulate autologous human tumor infiltrating lymphocytes (TIL).[16]

Treatment of human neuroblastoma cells with exogenous IFN-γ can increase expression of HLA class I antigens and ß-2 microglobulin, both *in vitro* and in patients.[17-19] Also, IFN-γ treatment of neuroblastoma cells *in vitro* increases their susceptibility to lysis by LAK cells and monocytes.[20,21]

The relationship between N-*myc* expression and MHC class-I expression in neuroblastoma has been investigated. In 33 neuroblastomas, amplification of N-*myc* appeared to correlate with low levels of MHC class-I expression.[22] Fusion of N-*myc* overexpressing human neuroblastoma cells with cells not expressing N-*myc* resulted in a decline in N-*myc* expression and expression of class-I MHC genes.[23] The apparent correlation between N-*myc* expression and expression of MHC class-I antigens is not absolute, however, and in a survey of 24 human neuroblastoma cell lines, N-*myc* expression was not invariably associated with low levels of ß2 microglobulin expression.[24] In studies of the MHC class-I promoter, distinct elements susceptible to N-*myc* mediated supression have been identified, and N-*myc* expression has been correlated with reduced binding of a transcription factor found to activate the MHC class-I enhancer.[25] This data, although inconclusive, suggests a relationship between N-*myc* expression and MHC class-I down regulation. We have introduced IFN-γ vectors stably into several patient derived neuroblastoma cell lines. Levels of IFN-γ production have ranged from 12 to 1550 u/ml and have persisted for up to 2 months *in vitro*. Transduction into the low MHC-I expressing LAN-6 cell line has increased MHC-I expression up to 1000 fold as measured by FACS analysis. Less striking increases in MHC-II expression have also been noted. In addition, upregulation of the neuroblastoma tumor associated antigen HSAN 1.2was noted in transduced SKN-AS cells. This suggests that IFN-γ will be highly useful in enhancing immunogenicity of neuroblastoma cells *in vivo*.

2.2 Adult Studies. There have been no publications dealing with immunization utilizing IFN-γ transduced human tumor cells. One protocol for patients with melanoma has been approved by the RAC and is open at Duke University. This protocol employs the same retrovirus for transduction of IFN-γ cDNA that we shall utilize (provided by Viagene, Inc.).

2.3 Pharmacology. Pharmacokinetics and biological activity in subcutaneous long-term administration of recombinant IFN-γ in cancer patients have been investigated.[26] In doses of 0.1, 0.25, or 0.5 mg/m^2 in two or three injections per week for up to 180 days there was limited intra-individual variability with respect to the plasma concentration/time profiles. However, the interindividual variation was high for the parameters area under the data points and maximum plasma concentration. Despite this high interindividual variability, the mean values obtained for were approximately proportional to the dose administered (0.1, 0.25 or 0.5 mg/m2). In this study, objective antitumour responses were observed in two patients, but there was no partial or complete remission.

Summary. We aim to maximize anti-tumor immune responses to neuroblastoma cells by increasing their immunogenicity through genetic engineering. Our large bank of neuroblastoma cell lines and matched normal leukocytes provides a unique opportunity to determine if partially MHC matched tumor cells can be effective immunogens. The availability of patients with measurable disease as well as no detectable disease after ABMT provides two distinct settings for testing the toxicity and immunogenicity of genetically engineered autologous and allogeneic neuroblastoma cells. The proposed studies are relevant to a variety of other high risk human tumors for which intensive, cytoreductive therapy followed by immunologic therapy may be an effective treatment.

3.0 PATIENT ELIGIBILITY AND STUDY ENTRY

3.1 Eligibility

Two groups of patients are eligible:
- *Group 1*: those who received carboplatin, etoposide, melphalan, and local irradiation (CEM-LI, protocol 91LA6) or other marrow-ablative therapy followed by ABMT approximately 100 days previously, who usually do not have detectable disease but who are at significant risk for recurrence.
- *Group 2*: those who have persistent or progressive disease.

3.11 Genetically engineered neuroblastoma cells. The patient must have available either autologous or one locus matched (unrelated) neuroblastoma cells that are transfected with IFN-γ vectors and that can be prepared in sufficient quantity for immunization according to RAC and FDA guidelines.

3.12 Immune status. Absolute lymphocyte count at least 1500/mm^3; T and B cell numbers, PHA response, within 3 SD of normal for age.

3.13 Previous therapy. No exclusions. Patients must have fully recovered from the toxic effects of any prior therapy. At least 2 weeks should have elapsed since the last dose of chemotherapy (4 weeks in the case of nitrosourea containing therapy).

3.14 Patients must have been less than 21 years of age when originally diagnosed to be treated on this protocol.

3.15 Patients must have had histologic verification of neuroblastoma.

3.16 Patients in Group 2 must have disease that is refractory to conventional therapy.

3.17 Patients must have a performance status of 0 or 1 (Appendix L).

3.18 Patients must have a life expectancy of ≥2 months.

3.19 All patients must have:

3.19.1 Adequate bone marrow function, defined as:
- Peripheral absolute neutrophil count (ANC) ≥1000/mm^3
- Platelet count ≥20,000/mm^3 (may receive platelet transfusions)
- Hemoglobin ≥8.0 gm/dl (may receive RBC transfusions)

3.19.2 Adequate renal function defined as:
- Serum creatinine ≤1.5 x normal, or
- Creatinine clearance or radioisotope GFR >40 ml/min/m^2 or >70 ml/min/1.73 m^2

3.19.3 Adequate liver function defined as:
- Total bilirubin ≤1.5 x normal, and
- SGOT (AST) or SGPT (ALT) <2.5 x normal.

3.194 Central nervous system function defined as:
- Patients with seizure disorder may be enrolled if seizures are well controlled by anticonvulsants.
- CNS toxicity ≤grade 2.

3.21 All patients and/or their legal guardians must sign a written informed consent.

3.22 All institutional, FDA, NCI, and RAC requirements for human studies must be met.

3.2 Study Entry Patients will be entered on study by telephoning (213) 669-4565 between 8:30 a.m. and 5:00 p.m. Monday through Friday, including holidays. The information requested on the Telephone Study Entry Form must be provided at the time of this telephone call. The dose level will be assigned by Drs. Seeger or Villablanca at the time of study entry.

4.0 TREATMENT PROGRAM

4.1 Treatment: Two groups of patients, as defined in 3.1, will be immunized. Immunizations will be given on weeks 0, 2, 4, 8, 12, 24, and 36 unless the patient is removed from the protocol (see 7.0).

4.2 Dose Escalation

4.21 **Inter-Patient Escalation:** A given patient will receive the same dose of cells at each immunization. Three patients will be entered per dose level for each group of patients (Groups 1 and 2).

Escalations are as follows:

level 1: 1×10^7 cells/m^2/dose
level 2: 5×10^7 cells/m^2/dose
level 3: 10×10^7 cells/m^2/dose

The potential systemic dose of IFN-γ over 4 days will not exceed 10% of its MTD when given parenterally. Each immunizing dose will be divided into two subcutaneous injections and alternately given below the inguinal and axillary areas. Unless toxic effects suggest otherwise, autologous and allogeneic vaccines will be considered the same for the purposes of dose escalation.

Table 1. Dose escalation schema.

No. Pts. with Events at Current Dose[1]	Change in Dose Level Based on No. Events
0/3	Advance to next higher level
1/3	Accrue three more patients, for total of 6 evaluable patients
1/6	Do not advance, do not decrease
2/6	Decrease dose to next lowest level
2/3	Decrease dose to next lowest level

[1]An event is a treatment related death or a grade 3 or 4 toxicity.
If known cytokine related toxicity is observed, the dose will be reduced to the previous level or, if necessary, discontinued.

4.22 Intra-Patient Escalation. There will not be intra-patient dose escalation.

4.3 Dose Limiting Toxicity; The MTD for IFN-γ transduced neuroblastoma cells will be defined as the highest dose that is tolerated by ≥2 of 3 (or 4 of 6) patients receiving that dose without evidence of grade 3 or 4 toxicity that is not reversible within 28 days of tumor cell injection (CCG Toxicity Rating Scale, Appendix L). The most common adverse effects from IFN-γ are flu-like symptoms, fever and fatigue.

4.4 Concomitant Therapy

4.4.1 No other systemic cancer therapeutic agents are allowed.

4.4.2 Radiotherapy to localized painful lesions is acceptable, provided at least one measurable lesion is not irradiated. Any irradiated lesion can not be used to assess tumor response.

4.4.3 Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary.

4.4.4 Corticosteroid therapy, local or systemic, for treatment of debilitating local or systemic immune reactions is permissible.

5.0 REQUIRED OBSERVATIONS

5.1 Clinical Assesment

5.11 Pretreatment Evaluation

5.11.1 History, physical examination, weight, vital signs, performance status;
5.11.2 CBC, platelet count, AST, ALT, bilirubin, PT, PTT, urinalysis;
5.11.3 Chest x-ray;
5.11.4 Tumor measurement (CT, MRI or ultrasound).

5.2 Evaluation During Treatment

5.21 Weekly physical examination, vital signs, weight, performance status weeks 0-4 and then as indicated with a minimum of monthly evaluations;
5.22 CBC, platelet count, AST, ALT, bilirubin, urinalysis before each tumor cell injection;
5.23 Tumor measurement (CT, MRI or ultrasound) on weeks 8, 24, and 36.

5.3 **During Follow-Up**: If the patient is on study after the week 36 injection, follow-up as below until the patient goes off study:

5.31 Monthly physical examination, vital signs, weight, performance status x6 and then every three months for three years;
5.32 Monthly CBC, platelet count, AST, ALT, bilirubin, PT, PTT, urinalysis x6 and then every three months for three years;
5.33 Tumor measurement every three months x 4 and then every 6 months.

6.0 DRUG INFORMATION

6.1 **Interferon-gamma transduced human neuroblastoma cells.** Each cultured human neuroblastoma cell line is given an anonymous designation (CHLA-cell line number) and each IFN-γ transduced line or subclone receives an additional IFN-γ designation. Thus, for example, a transduced cell line could be designated as follows: CHLA-100-IFN-3. This identifying data with appropriate descriptive and testing information will be maintained in a computerized database.

6.11 **Preparation of IFN-γ Transduced Neuroblastoma Cells.** Neuroblastoma cells from surgical, marrow, or blood specimens will be utilized to establish tumor cell lines. The resultant cell culture will be transduced with the IFN-γ retroviral vector construct, which will be provided by Viagene, Inc. The transduced tumor cells will be evaluated for IFN-γ, MHC class I and II, and B7 expression (See Appendix L). Cell lines (or subclones) that express class I antigens and IFN-γ will be identified for clinical use and subjected to standard testing according to FDA and RAC guidelines. (see Tables 1-5, Appendix L).

If possible, an autologous tumor cell vaccine will be prepared for each patient. However, if this is not possible, a single locus matched allogeneic tumor cell vaccine will be prepared. The relative frequencies of HLA-A and -B loci is not random, with unusually high representation of certain subtypes among different ethnic groups. As an example, over 49.4% of Caucasians, and 48.3 % of Asians, and 31.9% of Blacks express the HLA-A A2 antigen. Similarly, the HLA-B B44 antigen is seen in 23.0% of Caucasians, 11.6% of Asians and 14.8% of Blacks. Therefore, within our relatively large tumor bank (>100 cell lines), there may be a significant representation of certain HLA-A and -B subtypes, and even shared haplotypes may not be rare. Serologic (lymphocytes, bone marrow, neuroblastoma cells if HLA expressed) and genotypic (neuroblastoma cells) HLA typing will be done by Dr. P. Terasaki. Those tumors with frequently shared MHC class-I antigens (eg., A2, B3, B44) will be used to generate a library of IFN-γ producing cell lines that express MHC molecules. This library will include N-*myc* amplified and non-amplified cell lines so that appropriate biologic subtypes are available for allogeneic immunization. Hence, if certain neuroblastomas have common MHC antigens, they could serve as a presenting source of tumor cell derived

antigen in an appropriate MHC class-I context. This could lead to much more simplified tumor vaccination schemes and potentially alleviate the need for cytokine introduction into individual autologous tumor cells.

The retroviral vector used in these studies is based upon Moloney murine leukemia virus, which has been engineered to replace its extraneous viral coding sequences with the human IFN-γ cDNA. Unlike true viruses, the recombinant vector is incapable of replication since it lacks all the viral structural genes. A detailed characterization of the vector gene construct will be presented to the RAC and the FDA for approval prior to initiation of the study.

6.12 Formulation and Storage. IFN-γ transduced tumor cells are supplied frozen viably in L15 tissue culture medium with 2.5% human serum albumin, 3% Hespan, and 20% dimethylsulfoxide at concentrations appropriate to provide viable thawed cells for the dose levels indicated. For example, freezing 1.5×10^7 cells in 1 ml of medium would yield approximately 10^7 cells on thawing (anticipated recovery of 66%); this would provide sufficient cells for one course for a 15 kg patient (approximately 0.66 m^2) receiving the level 1 dose. Viably frozen cells will be stored in sealed, sterile tubes in liquid nitrogen vapor and only removed immediately before injection.

6.13. Stability. Once viably frozen, cells are stable indefinitely, provided they are not removed from liquid nitrogen vapor.

6.14 Preparation for Injection. Cells will be rapidly thawed by partial immersion of the vial in a 37 °C water bath. Once thawed, they will be washed once, assessed for viability, and resuspended at an appropriate concentration in 1 ml of L15 medium containing 2.5% human serum albumin. Without delay, thawed cells will be irradiated (5000 cGy) and injected into the patient.

6.15. Administration. The appropriate total cell dose will be divided into two aliquots and injected into two sites (alternate thighs with arms) subcutaneously. The volume of cells injected generally will be 0.25 - 0.5 ml in each site. The cell concentration will be $<50 \times 10^6$ per ml to prevent excess cell aggregation, and so it may be necessary to inject more than 2 sites for some patients.

7.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY

7.1 Any patient who develops progressive disease.
7.2 Any patient who develops unmanageable local or regional toxicity.
7.3 Any patient who develops grade 3 or 4 toxicity that does not resolve within 28 days.
7.4 Completion of 7 courses of therapy (weeks 0-36).
7.3 Allergic reaction to IFN-γ or life-threatening allergic reaction to IFN-γ transduced tumor cells.
7.4 At discretion of patient or physician.

8.0 SUPPORTIVE CARE

Supportive care will be given according to the judgement of the treating physician.

9.0 IMMUNOBIOLOGIC AND MARROW STUDIES

9.1 Patient Considerations. In addition to routine tests for evaluating toxicity, the following selected tests will be performed. These will provide information about the patient's immune status, immunologic response to the tumor cell injection, interferon level, and possible anti-tumor effect.

9.2 Sampling Schedule. The following studies will be done before and after immunization on weeks 0, 8, 24, and 36:

9.21 Blood. Absolute numbers of T cells, T cell subsets, and B cells; response to PHA.

9.22 Blood.: T cell and CD8+ cytotoxicity (direct and after *in vitro* expansion with cytokine producing, irradiated immunizing tumor cells) for autologous and allogeneic neuroblastoma cells.

9.23 Bone marrow. quantitative immunocytologic analysis for neuroblastoma cells involvement (only for patients with detectible metastases at study entry). Bone marrow examination should be bilateral aspirates, no biopsies needed.

9.3 Sample Preparation20 ml of heparinized blood (100 units preservative-free) will be obtained for 9.21 and 9.22. Approximately 2 ml of heparinized marrow will be obtained from bilateral iliac crests for 9.23. For tests of blood and marrow cells, mononuclear cells will be prepared by density sedimentation using standard methods.

10.0 STATISTICAL CONSIDERATIONS (37)

This trial will be a Phase I study of IFN-γ transduced neuroblastoma cells administered by subcutaneous injection. The starting dose of cells will be $1 \times 10^7/m^2$. Two successive dose levels will increase the cell dose to 5×10^7 and $10 \times 10^7/m^2$. A minimum of 3 evaluable patients who have not previously received this regimen will be entered at each dose level (see Section 4.2). Review of entry rate into previous phase I studies of high risk neuroblastoma indicates that sufficient numbers of patients are available from the three participating institutions to complete the study within 24 months.

Endpoints are MTD (may not be reached) or progression of disease. Toxicity will be defined as that related to the cytokine or to the site of injection.

The MTD will be defined as the highest dose that is tolerated by ≥ 2 of 3 (or 4 of 6) patients receiving that dose without evidence of grade 3 or 4 toxicity as defined by the CCG Toxicity Rating Scale that is not reversible within 28 days of stopping the treatment. Toxicity will be assessed during each treatment period using standard criteria (history, physical examination, laboratory tests).

Laboratory correlates will be determined as described above. Results for Group 1 and 2 patients will be compared.

Disease status will be determined for all patients at study entry and repeated at weeks 8, 24, and 36.

Nine patients will be entered for each set of engineered cells (unless toxicity dictates more): three patients per dose level from both Groups 1 and 2. Each Group will be analyzed separately since MTD and laboratory parameters may be influenced by ABMT. It is anticipated that 1.5-2 years will be required to complete the phase I/Ib study for each set of engineered tumor cells.

11.0 ADVERSE REACTION REPORTING AND TOXICITY CRITERIA

11.1 Guidelines for Reporting Adverse Drug Reactions; The timely reporting of adverse reactions (including toxic deaths) is required by the Food and Drug Administration. Reactions definitely felt not to be treatment related should not be reported; however, a report should be submitted if there is any suspicion of treatment-related effect. The reporting of adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the data reporting for this study.

11.1.1 Reporting adverse drug reactions occurring with Investigational Agents; -Within 24 hours of recognition, contact the study Co-Chairman (Dr. Seeger or designee) by telephone to verify the identification process and the existence of an adverse drug reaction or toxic death.
-The IND for the investigational agent will be jointly held by Dr. Joseph Rosenblatt (UCLA) and Dr. Robert Seeger (CHLA).
-Upon concurrence by the Study Co-Chairman (Dr. Seeger), report the following data:

Institution
Patient identification number
Presenting diagnosis
Laboratory data supporting event
Total dose(s) of suspected drug
Time to event
Type and grade of adverse event
Methods used to recognize and characterize the effect

-Within seven (7) days a completed NCI Adverse Reaction Form for Investigational Agents should be sent to the study Co-Chairman (Dr. Seeger) for all life-threatening events which may be due to drug administration, all fatal events while on protocol therapy, or first occurrence of any **previously unknown** clinical event (regardless of grade). The study Co-Chairman will forward this form to the FDA within ten (10) days of the adverse reaction.

11.1.2 Childrens Hospital Los Angeles (CHLA), Division of Hematology-Oncology (H-O);
-CHLA/H-O will file all adverse drug reaction reports with the FDA within ten (10) days of the adverse drug reaction. A copy of all reports will be sent to the Study Committee.
-CHLA/H-O will coordinate actions to describe patient performance and compliance issues associated with treatment. This action will be initiated to determine needs for study modification. Upon indication, trials will be closed by the Study Chairman until analyses and evaluations are complete.

11.2 Toxicity Criteria The CCG Toxicity Rating Scale is provided in Appendix III. Toxicities are to be reported on the appropriate data collection forms.

12.0 RECORDS AND REPORTING

12.1 Theradex Forms; Data for this study will be collected using the case report forms of Theradex, Inc. All data forms are to be provided to Dr. Seeger at the intervals specified below. The manual describing conventions for the completion of the case reports is available at CHLA/H-O.

12.11 Filing Schedule
12.11.1 History and Physical - within 7 days of telephone study entry.
12.11.2 Prior Therapy -within 7 days of telephone study entry.
12.11.3 Concomitant Measures - within 7 days of patient's final dose of IFN-_ transduced neuroblastoma cells.
12.11.4 Study Drug Administration - within 7 days of patient's final dose of IFN-_ transduced neuroblastoma cells.
12.11.5 Physician Notes - within 7 days of cessation of protocol mandated therapy.
12.11.6 Course Assessment - within 7 days of the end of each course of protocol mandated therapy.
12.11.7 Off Study Summary - within 7 days of the cessation of protocol mandated therapy.
12.11.8 Flow Sheets - a set of flow sheets must be completed every 4 weeks while the patient is receiving protocol mandated therapy. The flow sheets must be mailed to Dr. Seeger's every four weeks.
12.11.9 Pharmacokinetics and Urinary Excretion Form - Not applicable to this study.

12.2 CCG Forms
12.2.1 Specimen Transmittal Form
This form is required with submission of blood and none marrow specimens.
12.2.2 Disease Status and Event Form
This form is to be submitted at yearly intervals, after a patient goes off protocol therapy, until the patient enters another therapeutic study, dies, or is lost to follow-up.
12.2.3 Death Registration Form
This form is designed to capture information regarding the death of a patient and is to be submitted to Dr. Seeger within two weeks of a patient's demise.

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Attachment II

CHILDREN'S ASSENT FORM

I have been asked to take part in a study designed to test a new treatment for neuroblastoma, a type of childhood tumor. My doctors have told me that I have neuroblastoma, and that (1) although there is no evidence for disease now, there is a possibility the disease may return, or (2) that there is still tumor left after usual treatment. I am, therefore, being asked to try a new experimental treatment to help destroy remaining cancer cells. The treatment is called gene therapy. My cancer cells, or cancer cells from another patient with neuroblastoma, have been growing in the laboratory and have been specially altered to make gamma interferon, a substance which may help my immune system recognize and fight the cancer cells. If I agree to take part in the study, I will receive injections containing tumor cells that have been treated with radiation to prevent them from growing, under the skin, seven times in nine months. My doctors will follow me carefully to see if I have any bad or good effects from the injections. They hope the injections will "teach" my own immune system to destroy tumor cells.

They will examine me every week for four weeks and every month after four weeks. They will draw blood before every tumor cell injection and will obtain several special tubes (20 ml — four teaspoons of blood) to find out how my immune system is doing before the first injections, and at week 8, week 24 and week 36.

It is possible that I may have some bad effects called "side effects" from these injections. I may feel pain where the injection is given, a sore may form at the site, I may develop a skin rash, fever, chills, muscle aches, or feel tired. There may be other side effects that I cannot be told about in advance, because this type of treatment has not been tried before in people.

My doctors hope that this therapy will kill my tumor cells, but the therapy may not work.

If I have any questions, I may call Dr. Joseph Rosenblatt, at (310) 825-2745, or my treating physician at any time.

I may keep a copy of this assent form if I like.

_____ _____

Date Informant (Investigator)

_____

Your Signature

Date of Expiration: January 6, 1995

Attachment III

INFORMED CONSENT

Study Title: A Phase One Study of Immunization with Gamma Interferon Transduced Neuroblastoma Cells

Principal Investigators: (Robert C. Seeger, M.D., Co-Chairman, Children's Hospital, Los Angeles; Joseph D. Rosenblatt, Co-Chairman, UCLA)

Stephen Feig, M.D., Oncologist, UCLA

Randal Wada, M.D., Oncologist, UCLA

Introduction and Purpose

I (my child/ward) hereby authorize Dr. Joseph Rosenblatt, M.D., Dr. Stephen Feig and Dr. Robert Seeger, M.D. and their associates _____

_____

to include me (my child/ward) in the research project entitled A Phase One Study of Immunization with Gamma Interferon Transduced Neuroblastoma Cells.

I have been asked to participate in a research study which is designed to test the safety of administering neuroblastoma cells to me (my child/ward), that have been genetically engineered to express gamma interferon, a substance known to enhance the immune response in certain tumor models, and to see whether this treatment can provoke an effective anti-tumor immune response by increasing the ability of the immune system to recognize and destroy neuroblastoma cells. I (my child/ward) have been asked to enroll in this study because I (my child/ward) have neuroblastoma which has been treated by high dose chemotherapy and autologous bone marrow transplant but which still may have a significant likelihood of recurring, or, because the neuroblastoma has been treated with conventional therapy but this has failed to totally eradicate the tumor cells, and persistent or recurrent neuroblastoma is present.

The aim of this phase I study is to determine the safety of administration of genetically engineered neuroblastoma cells when given as an injection under the skin, and to determine the maximum tolerated dose of such cells so that in future studies the doses required for maximum potential effect will be known. No effect of these tumor cell preparations has been demonstrated yet in human cancers.

New types of therapy which utilize the immune system to help fight the cancer may be able to get rid of tumor cells that may have remained after conventional treatment with chemotherapy, bone marrow transplant and or radiation, or may also be useful in treating tumor which has remained following standard therapy. I understand that I (my child/ward) will be treated with a new tumor cell preparation that is administered by injection under the skin. The tumor cell that we will use is made by taking neuroblastoma cells from me (my child/ward) or from another child with neuroblastoma by biopsy of a tumor mass, or bone marrow aspiration/ In the laboratory the tumor cells are altered by putting in new genetic material (DNA) which is expressed in the tumor cell. The tumor cells now make a protein called gamma interferon which is thought to activate the immune system. It is hoped that gamma interferon produced by the tumor cells might "turn on" the immune system, so that it may potentially fight against tumor cells left in the body. The DNA that makes gamma interferon is put into tumor cells by infecting them with a weakened mouse virus called a retroviral vector which carries this DNA. This virus has been altered in the laboratories so it should not grow further or cause a viral infection in other cells after being inserted into the tumor cells. Tumor cells which carry the new DNA will be irradiated with x-rays before being injected into the skin so that they will no longer be able to grow. It is hoped that after injection they might act to increase the body's immune reactivity to the tumor resulting in the killing of tumor cells remaining in my (my child/ward's) body.

The purpose of this study is to:

1. Determine the maximal tolerated dose and side effects of the genetically engineered tumor cell treatment.

2. To provide information which is helpful in the development of new and similar treatment programs in the future.

To determine the maximal tolerated tumor cell dose, the treatment will start at a low dose thought to be tolerable. If tolerated, the dose will then be increased for the next group until significant side effects are seen or until the maximal dose envisioned in this study is being administered. Each patient will however, receive a preset dose of cells.

Procedures

The genetically engineered tumor cell treatment is made in the laboratory under sterile conditions from neuroblastoma cells found in the blood, bone marrow or biopsy of a tumor from me (my child/ward) or another child with neuroblastoma, if my (my child/ward) own

neuroblastoma cells are unavailable or did not grow in the laboratory. The tumor cell preparation is tested to determine that it is free of known harmful infectious agents. The irradiated tumor cells will be injected under the skin at two or possibly more sites, either in the upper arms or thighs on one day in week 0, and one weeks 2, 4, 8, 12, 24 and 36. I (my child/ward) will receive injections over a 36 week period and followed for life. Because gene therapy of this type is a novel form of anticancer therapy, I (my child/ward) will be followed through my (my child/ward's) life, and contacted at least annually to see how I (my child/ward) is doing. I (my child/ward) will be asked to maintain contact with the investigators and to provide a new address if I (my child/ward) moves. If I (my child/ward) die for any reason, either during or after my participation in this study, permission to perform an autopsy will be sought.

Irradiated tumor cells will be given in the pediatric outpatient clinic unless significant side effects occur which require a closer monitoring in the hospital. Treatment will be stopped if severe side effects occur or if the tumor continues to grow despite administration of the treatment.

Required Tests

A number of examinations will be necessary before, during and after this treatment program if I (my child/ward) agree to enroll in this study. These include usual blood tests to evaluate normal organ function and special blood tests to measure my immune function or that of my child/ward. The blood test done to measure immune function will be done at study entry and at weeks 8, 24 and 36. Immune function tests will require the withdrawal of approximately 20 cc's (four teaspoons) of blood. All blood tests will be drawn by vein or through a venous access device such as a catheter if one is available. An additional teaspoon of blood will also be drawn before the vaccine is given and forty-eight hours later on weeks 0, 8, 24 and 36 to determine the blood level of gamma interferon which is being made by the injected tumor cells. Other tests to be done during this treatment program include scans and radiologic evaluations to follow my (my child/ward) disease status. I (my child/ward) will have a bone marrow aspirate and biopsy performed on both sides of the hip at study entry. This procedure may cause some pain/discomfort and will be explained to me (my child/ward) separately by our doctors. Periodic repeat bone marrow examinations will only be done if my (my child/ward's) bone marrow test was positive for neuroblastoma at the time of entry in the study. All these tests are needed to measure my (my child/ward's) clinical condition, progress and immunity to the tumor cells. If any tumor cells are obtained from me (my child/ward) during necessary

follow-up tests, they may be used for research purposes including the growing of additional neuroblastoma cells in the laboratory, unless indicated otherwise.

Potential Risks

Administration of tumor cells genetically engineered to express interferon has never been studied in humans before and may have unknown side effects. These include damage to normal tissues in the area of administration. Problems which arise are likely to be reversible when the treatment is stopped but occasionally can persist and may cause serious subsequent complications. The following kinds of side effects could be observed with this treatment program:

1. Allergic reactions such as rash, skin tenderness, swelling, fever and or hives. More severe allergic reactions including shortness of breath, a drop in blood pressure and or fainting could also be possible.

2. Pain, bruising and or infection may occur at the injection sites. Granuloma formation (development of hard lump) at the injection site could occur. The granuloma could become hardened, red and irritated and could require a skin incision to drain it by my (my child/ward's) physician. It could be present as long as I (my child/ward) continue to receive tumor cell injections, but should likely resolve within several weeks of stopping treatment.

3. I (my child/ward) could experience general malaise, lethargy, joint pains and/or fever. The treatment could also cause my (my child/ward's) immune system to attack tissues within my (my child/ward's) body. Such immune attack is known as an autoimmune reaction. This could result in symptoms such as rashes, sores in the mouth, fever, joint pains, enlargements of lymph glands, abnormalities of kidney function and or liver function, and potentially in abnormalities of the nervous system which are presently unknown.

4. There is a small possibility that the tumor cell preparation could harbor a virus which might cause a second cancer to develop in me (my child/ward). This cancer would likely be a different cancer from the neuroblastoma. Precautions are taken to avoid transfer of viruses that might be likely to result in a second cancer, but it is not possible to completely insure against the possible transfer of viruses. Although highly unlikely, it is also theoretically possible that tumor cells from the treatment might themselves survive in the body and form a tumor. They have been treated with x-rays to prevent this.

5. Since this tumor cell preparation has never been previously tested in humans there could be toxic effects that could occur of which we are currently unaware.

Potential Benefits

There is no guarantee that this treatment will have any beneficial effects against my (my child/ward's) tumor. The potential benefit of the program is to inhibit the growth and spread of neuroblastoma, but the response of any patient may vary and I (my child/ward) might not respond to this treatment program. As a phase one study, this study is primarily designed to determine the maximum tolerated dose of the treatment that is still found to have manageable side effects. Therefore, it is possible that you (your child/ward) might receive a dose of tumor cells below the dose which may have the most tumor action. Considerable knowledge should be gained from this study program to allow us to design future studies involving the use of genetically engineered tumor cells, and this knowledge may help you (your child/ward) and/or other children by improving the treatment of this and other cancers if the future. It is also quite possible that you (your child/ward) will receive no direct benefit from participation in the study.

Alternative Therapies

This program uses a new experimental approach for the treatment of neuroblastoma. The alternative is to continue with other investigational therapies such as alternative forms or combinations of chemotherapy, and/or radiation, or to give no further therapy other than supportive care. These options, including supportive care to keep me (my child/ward) comfortable, will not be affected by a decision to enter or withdraw from this treatment program.

Right to Refuse or Withdraw

I understand that I (my child/ward) have the right to refuse to participate and/or withdraw from this research at any time without prejudice to my (my child/ward's) future care at UCLA. I understand that circumstances may arise which might cause the investigator to terminate my (my child/ward's) participation before completion of the study. The investigator will provide me with new information developed during the course of the study that might influence my desire to continue my participation.

Costs

I understand that I and my insurance carrier are financially responsible for all x-rays, laboratory tests, hospital and or physician fees required for routine clinical care/follow-up during the course of the study. The tumor cell preparation will be prepared and genetically engineered to

express gamma interferon free of charge. Specialized immunological tests, as well as testing of levels of gamma interferon production levels will be performed at no charge to you. There will be no charge for the genetically engineered tumor cell preparation itself, nor for fees involved in its administration.

Confidentiality

I (my child/ward) have a right to privacy, and all information that is obtained in connection with this study and that can be identified with me (my child/ward) will remain confidential as far as possible within state and federal law. My (my child/ward's) identity will not be disclosed to the press without prior consent on my (my child/ward's) part. The results of this study may be published in a scientific journal or book, without identifying me (my child/ward) by name.

Records will be kept regarding my (my child/ward's) participation in the study and will be made available for review only as required by the Food and Drug Administration under the guidelines established by the Federal Privacy Act.

I understand that the Food and Drug Administration, the National Institute of Health and Viagene, Inc. are permitted to have access to my medical record and to the data produced by the study, for audit purposes. However, they are required to maintain confidentiality.

Other Information

Cells obtained from my (my child/ward) blood may be used to establish a cell line which may be shared in the future with other researchers and which may be of commercial value. A cell line is one which will grow indefinitely in the laboratory. Cell lines might be useful because of the characteristics of the cells and/or the products they might produce.

I (my child/ward) relinquish any and all commercial/monetary rights I (my child/ward) have in the blood and tissue samples I (my child/ward) will donate as part of this study, as well as any rights to any research using these blood and tissue samples or their by-products, and to any discovery or product arising from the use of my donated blood samples or any part, derivative or product of the blood samples.

If I (my child/ward) am of childbearing potential, due to the possible unknown risks to a fetus, I (my child/ward) will not participate in this research study unless with the investigator's knowledge and approval, I (my child/ward) am using a medically accepted form of barrier birth control (contraception).

I understand that if I am (my child/ward is) injured as a direct result of these research procedures I (my child/ward) will receive medical treatment for those injuries at no cost. The University of California does not provide any other form of compensation for injury.

I understand that Joseph Rosenblatt, M.D., who can be reached at (310) 825-02745, or _____or Robert C. Seeger, M.D., who can be reached at (213) 669-5618 will answer any questions I may have at any time concerning the details of the procedures performed as a part of this study.

I understand that if I (my child/ward) have further questions, comments or concerns about this study and or informed consent process, I may write or call the office of the Vice Chancellor of Research Programs, 2138 Murphy Hall, UCLA, Los Angeles, California 90024-1405, (310) 825-8714.

In signing this consent form I acknowledge receipt of a copy of the form as well as a copy of the subject's bill of rights.

_____
Subject's signature

Date:_____

_____
Witness

Date:_____

The subject is a minor (age _____), or is unable to sign because _____
_____
_____

_____
Mother

Date:_____

_____
Father

Date:_____

_____
Guardian

Date:_____

CHILDRENS HOSPITAL LOS ANGELES

INFORMED CONSENT

A CLINICAL STUDY PROGRAM USING HUMAN GAMMA INTERFERON TRANSDUCED NEUROBLASTOMA TUMOR CELLS TO IMMUNIZE PATIENTS WITH HIGH RISK NEUROBLASTOMA WITH PERSISTENT OR PROGRESSIVE DISEASE

BACKGROUND AND PURPOSE

Children with neuroblastoma that is very difficult to treat ("high risk neuroblastoma"), who have persistent or new tumor growth ("progressive disease") during or following treatment are eligible for participation in this experimental treatment program (a phase I study). Neuroblastoma cells that have been modified to make gamma interferon will be injected under the skin in an attempt to stimulate the immune system. The main goals of this study are to determine 1) the dose of modified neuroblastoma cells that can be injected without serious side-effects; 2) the response of the immune system to the injected tumor cells. Although we hope that this immunologic therapy will stimulate the immune system to kill tumor cells, there is no guarantee that this will occur.

You (your child)_____ have (has) high risk neuroblastoma that was diagnosed before 21 years of age. Nearly all children with neuroblastoma that has persisted or regrown despite treatment with chemotherapy, radiation, and surgery do not survive their disease. The immune system, if properly stimulated, may be able to kill tumor cells. Therefore, we are testing whether a response against the tumor is stimulated by neuroblastoma cells that have been modified to make gamma interferon. The first question to be answered in this clinical study is whether injecting modified neuroblastoma cells causes severe side effects. We are offering you (your child) participation in this study because you (your child) still have(has) tumor cells that are growing. The results of this study will provide information helpful in the further development of immunologic treatments for neuroblastoma.

Neuroblastoma cells used in this study are obtained from a tumor mass, bone marrow, or blood of you (your child) or another child and grown in the laboratory. Next, they are modified by putting in DNA for the human gamma interferon gene. A gene is a piece of DNA that is found inside all cells and that carries information for a cell to make a protein product. Neuroblastoma cells that have been given the gene for gamma interferon make gamma interferon protein, which can change them and possibly activate the immune system. The DNA that makes gamma interferon is put into the tumor cells by infecting them with a weakened mouse virus which carries this DNA. This virus (called a vector), which is produced in the laboratory by cells from a dog, has been weakened so that it will not grow and cause a viral infection in other cells besides the tumor cells. The tumor cells that carry the new gamma interferon DNA are irradiated so they will not grow and form tumors. Then they are injected

under the skin, where we hope they will activate the body's immune system to kill tumor cells that remain in your (your child's) body.

The number of modified tumor cells that can be injected without serious side effects will be determined by giving the first group of patients a dose thought to be tolerable based upon experience with injecting other types of tumor cells. If this first dose does not cause side effects, a second higher dose will be tested. Since this study begins with a relatively low dose of tumor cells for safety reasons, you (your child) may not receive the highest dose that has manageable side effects and that has the most anti-cancer action.

POTENTIAL BENEFITS

The potential benefit of this treatment is that it may kill neuroblastoma tumor cells in your (your child's) body. However, the response of patients may vary, and your (your child's) tumor may grow during or after this treatment program. Knowledge gained from this clinical study may help other children by improving the treatment of cancer in the future.

STUDY PLAN

The gamma interferon modified neuroblastoma cells will be prepared in the laboratory under sterile conditions. If possible, tumor cells from you (your child), will be prepared; if not, those from another child with neuroblastoma will be used. These cells will be tested to insure that they are free of known harmful infections.

The modified tumor cells will be injected under the skin at two sites (either the upper arms or thighs) on one day at week 0, 2, 4, 8, 12, 24, and 36. This will be done in the outpatient clinic unless side effects occur which require closer monitoring of you (your child) in the hospital. Injection of modified tumor cells will be stopped if severe side effects (toxicities) occur, and you (your child) will be treated with supportive care until the toxicity resolves. At that time, options of other experimental treatments or no further therapy will be discussed with you (your child), but no further injections of gamma interferon modified neuroblastoma cells will be given. If tumor grows while you (your child) are receiving this therapy, then you will be offered these same options.

Whether you (your child) do or do not (does or does not) complete the planned number of injections, life-long contact will be maintained with you to obtain information about possible effects of this treatment. If, unfortunately, you (your child) pass(es) away, an autopsy will be requested to obtain information about possible effects of this treatment.

REQUIRED TESTS

A number of examinations will be necessary before and periodically during this treatment program. These will include usual blood tests to evaluate normal organ

function and special blood tests to measure the immune function of you (your child). The blood tests done to measure immune function will be done at week 0 (before the first injection) and at weeks 8, 24, and 36. The immune function tests require approximately 20 cc (4 teaspoons) of blood from you (your child). All blood tests are drawn by vein or through a venous access device. Other tests done during this treatment program include scans and radiologic evaluations to follow disease status. All patients will have a bilateral (from both sides) bone marrow aspirate at week 0, before the first injection. Repeat bone marrow examinations will be done if needed to care for you (your child) or at weeks 8, 24, and 36 if your (your child's) bone marrow test was positive for tumor cells at study entry. All these tests are needed to measure your (your child's) clinical condition and progress.

If any tumor cells are obtained from you (your child) during necessary follow-up tests, they may also be used for research purposes, including growing them continuously in test tubes (cell lines), unless you (your child) request otherwise.

POTENTIAL RISKS

Injecting gamma interferon modified neuroblastoma cells can possibly cause damage to normal tissues. These unpleasant or harmful side effects are called toxicities. Adjustments of dosage and schedule may be possible to avoid severe side effects. Problems which do arise are usually reversible when the treatment is stopped, but occasionally can persist and may cause serious complications. The following kinds of side effects or problems may occur with this treatment program.

1. Allergic reactions such as a rash, skin tenderness, swelling, fever or hives. More severe allergic reactions include shortness of breath, low blood pressure or fainting.

2. Pain, bruising or infection at the injection site(s).

3. Decreased energy, fatigue, joint pains, and fever.

4. Immune system attack on your (your child's) own body (autoimmune reaction). This may cause symptoms such as skin rashes, fever, joint pains, enlarged lymph glands, and abnormalities of the nervous system, the kidneys, and the liver.

5. Tumor cells obtained from you (your child's) own body will be used if possible, since they may work the best to activate your (your child's) immune system against your (your child's) own tumor cells. However, if gamma interferon modified tumor cells can not be prepared from you (your child), then another child's neuroblastoma cells will be used. It is possible that neuroblastoma cells from another child will not work as well.

6. There is a rare possibility that injection of gamma interferon modified neuroblastoma cells could cause a second cancer to develop in you (your child).

This second cancer would be a different cancer from neuroblastoma.

7. There is a rare possibility that the modified tumor cells could grow in your (your child's) body, and become a neuroblastoma tumor. To prevent this, tumor cells are treated with radiation before they are injected into you (your child).

8. There is a rare possibility that a hardened, red, and irritated lump (granuloma) may form at the injection site, and that this may require a cut of the skin to drain. If it occurs, the lump should resolve within a few weeks after stopping therapy.

9. The effect of this treatment on bearing children is not known. Although patients who have previously received chemotherapy and irradiation may be unable to have children, those who participate in this study who of childbearing age will need to use birth control measures until three months after the last injection of modified tumor cells.

It is also possible that injecting gamma interferon modified tumor cells could cause side effects that we currently do not know about since this treatment has not been previously tested in humans.

ALTERNATIVES

This program is testing a new experimental approach that may kill neuroblastoma cells that have grown despite more conventional therapies. The alternatives are observation, with no further therapy, or other investigational therapies. Supportive care to keep you (your child) comfortable is always available and will not be affected by a decision to enter or withdraw from this treatment program.

PHYSICAL INJURY

The possible risks associated with this study plan have been presented. There is always the possibility that physical injury may occur. The Childrens Hospital Los Angeles maintains professional liability insurance to protect patients from financial losses resulting from physical injury caused through the fault of the hospital, its employees or its agents. If physical injury occurs in the absence of such fault, the hospital does not provide reimbursement for treatment expenses or other compensation for physical injury suffered as a result of your(your child's) participation in this study.

If you believe that physical injury has been suffered as a result of participation in this study, you(your child) should contact Dr. Robert Seeger at (213) 669-5618. Treatment for such injury will be available under the same financial arrangement as treatment is usually provided.

CONFIDENTIALITY

Case histories, the results of treatment, laboratory and pathologic data, photographs and x-rays may be published for scientific purposes, but neither your child's or your identity will be disclosed and confidentiality will be maintained. The Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS) and/or their designate(s), the company manufacturing the vector (the weakened virus that carries the DNA for gamma interferon), and appropriate personnel of Childrens Hospital Los Angeles may inspect the records relating to your (your child's) participation in the study. If the study plan or use of the information is to be changed, you will be so informed.

VOLUNTARY PARTICIPATION

This study program has been reviewed, and its consent form has been approved by the Committee on Clinical Investigations (also called the IRB-Institutional Review Board). The approval of this Committee does not alter the fact that the final decision to enter yourself (your child) in the study program is yours. Refusal to participate in this study will involve no loss of benefits or penalize your (your child's) care. You have the right to remove yourself (your child) from the study at any time. Your attending physician, Dr._____, will answer any questions you may have regarding the study. Subsequent medical care at the Childrens Hospital Los Angeles is in no way contingent upon participation in this study program.

HOW TO OBTAIN INFORMATION

Daytime Monday through Friday, 8 a.m. to 5 p.m., you may call(213) 669-2121. You may leave a message with a secretary, and a doctor will return your call. Evening, nighttime, and weekends or holidays: You may call the hospital number (213) 660-2450, and ask for the on-call doctor for the Hematology-Oncology Service. You may be asked to leave a message with the page operator, and the physician on-call will return your call as soon as possible.

If you have questions regarding your(your child's) rights as a patient with regard to this study program, you can contact Ms. Gwenn Oki, Administrator of the Committee on Clinical Investigations(IRB) at (213) 669-2265.

SIGNATURE

Your signature below indicates that you have read this document, understand its meaning; have had a chance to ask questions; have these questions answered to your satisfaction; and consent to your(your child's) participation in this study program.

You hereby give your consent for yourself(your child) to be treated according to this clinical study program. You have been given a signed copy of this consent form.

_____ _____
(Parent/Guardian) (Physician)

_____ _____
(Parent) (Date)

_____ Consent obtained by:
(Patient)

_____
(Witness)

_____
(Translator)

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Joseph D. Rosenblatt	Assistant Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
California State Univ., Northridge		1973	
Univ. of Calif., Los Angeles	B.S..	1976	Chemistry
UCLA School of Medicine	M.D.	1980	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1992-present	Associate Director, UCLA AIDS Institute
1987-present	Assistant Professor of Medicine, Division of Hematology-Oncology, UCLA
1986-1987	Instructor of Medicine, Division of Hematology-Oncology, UCLA
1983-1986	Fellowship, Division of Hematology-Oncology, UCLA
June 1983	Internship and residency, Department of Internal Medicine, UCLA Medical Center, Los Angeles.

Diplomate, American Board of Internal Medicine, Subspecialty in Medical Oncology, subspecialty in Hematology
Fulbright Scholarship, 1989-1990

Publications Selected from a total of 58 publications:

Rosenblatt JD, Zeltzer P, Portaro J, Seeger R: Inhibition of Antibody Dependent Cellular Cytoxicity by Protein A from Staphylococcus Aureus. J Immunol 118:3, 1977.

Chen ISY, Slamon DJ, **Rosenblatt JD**, Shah NP, Quan SG, Wachsman W: The *x* gene is essential for HTLV replication. Science 229:54-58, 1985.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME: Seeger, Robert C. MD

POSITION TITLE: Principal Investigator

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Willamette University, Salem, OR	BA	1962	Chemistry
Univ. of Oregon Med. School, Portland, OR	MS, MD	1966	Anatomy; Medicine
Natl. Inst. of Health, Bethesda, MD	Clin Assoc	1968	Cellular Immunology
Univ. College London, London, England	Postdoc. Fellow	1972	Tumor Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1966-1968 Pediatric Internship and Residency, University of Minnesota Medical School, Minneapolis, MN

1974-1977 Assistant Professor, Division of Immunology and Allergy, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA

1977-1981 Associate Professor, Division of Immunology and Allergy, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA

1981-1983 Associate Professor and Chief, Division of Cancer Immunology and Biology, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA

1983-1989 Professor and Chief, Division of Cancer Immunology and Biology, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA

1985-1989 Vice Chairman for Academic Affairs, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA

1986-1989 Director for Clinical Research, Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA

1989-present Professor and Deputy Division Head for Research, Division of Hematology-Oncology, Department of Pediatrics, Childrens Hospital Los Angeles/USC School of Medicine, Los Angeles, CA

HONORS: Special Post Doctoral Fellow, National Cancer Institute, 1972-1974
Research Career Development Award, National Cancer Institute, 1975-1980

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HUMAN GENE TRANSFER PROTOCOL

9403-069 Walker, Robert; NIH, Bethesda, Maryland; *A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T-Lymphocytes in HIV-Infected Identical Twins.*

Date of RAC Approval: 3-3-94
Date of NIH Approval: 8-23-94

Protocol Title: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T Lymphocytes in HIV-Infected Identical Twins

Scientific Abstract: The cell-mediated immune response plays an essential role in the host's defense against viral infection. Studies of cytomegalovirus (CMV) and influenza virus for which small animal models are available have revealed that CD8+ cytotoxic T lymphocytes (CTLs) represent the major component of this cellular immunity. Although optimal animal models for HIV infection await development, evidence that CD8+ CTLs represent the major and earliest immune response to HIV infection is supported by correlative data from HIV-infected patients. The clinical data available suggest that a breakdown of the host cell-mediated immune response may be responsible for progression to symptomatic AIDS. *In vitro* studies have not only confirmed that HIV-specific CD8+ T cells exhibit cytolytic activity toward HIV-infected targets, but have also revealed that CD8+ T cells have the ability to inhibit replication of HIV in lymphocyte cultures. Data supportive of the central role of CD8+ T cells in HIV infection suggest that adoptive transfer of HIV-specific CD8+ T cells may have potential as an immunotherapy for HIV-infected individuals.

Cell Genesys, Inc. has designed universal (HLA-unrestricted) chimeric T cell receptors (URs) that can redirect the antigenic specificity of peripheral blood mononuclear cell (PBMC)-derived CD8+ T cell populations to recognize HIV antigen(s) of choice expressed on the surface of infected cells. Upon binding to viral antigen, these URs initiate T cell activation, resulting in induction of effector functions including cytolysis of the virally-infected cell. The URs developed to date are chimeric receptors composed of antigen recognition and signaling domains. The UR proposed for initial clinical investigation is composed of the extracellular domain of the human CD4 receptor that recognizes the gp120 moiety of HIV env fused to the cytoplasmic domain of zeta, responsible for signal transduction in T cells. Using retroviral-mediated transduction with replication-defective retroviral vectors, we can routinely generate CD8+ T cells stably expressing high levels of HIV-specific URs. The UR+ CD8+ T cell population exhibits highly efficient cytolytic activity against T cells infected with HIV-1. In addition, preliminary findings show inhibition of viral replication of a low passage lymphocytotropic strain of HIV-1.

In a collaborative clinical study between Cell Genesys, Inc. and investigators at the NIH, we will assess the safety and tolerance of adoptive transfer of anti-HIV cytotoxic, syngeneic peripheral blood T lymphocytes containing the CD4-UR into patients with HIV infection. The donors for the CD8+ T cells will be HLA-identical seronegative twins of the HIV-positive patients. The proposed study is an open-label, comparative, sequentially randomized treatment with genetically unmodified or modified *ex vivo*-expanded T lymphocytes. The study is divided into two treatment periods. In the initial period, single doses of genetically unmodified T lymphocytes or single, escalating doses of genetically modifed T lymphocytes will be administered. In the second period, multiple doses of the maximum tolerated cell dose will be administered. By monitoring functional immune status, viral burden, clinical symptoms, organ function, and persistence of circulating gene-modified T lymphocytes, we hope to determine whether this potential therapeutic approach is feasible and safe. This study may form the baseline for future protocols using lymphocytes obtained directly from HIV patients.

Protocol Title: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T Lymphocytes in HIV-Infected Identical Twins

Non-technical Abstract: HIV infection progressively destroys the human immune system and results in AIDS in the majority of patients. There is currently no cure for HIV infection or AIDS. White blood cells called CD8+ T cells kill cells infected with viruses and are an important component of the body's defense against viral infections. Although CD8+ T cells play an important role in temporarily controlling HIV infection, data suggest that a breakdown of the cell response may be responsible for progression to AIDS. Cell Genesys, Inc. has designed "universal receptors" that when inserted into CD8+ T cells will recognize HIV-infected cells and kill them.

In the proposed clinical study, CD8+ T cells will be removed from an uninfected identical twin of an HIV-infected patient and modified by genetically inserting the"universal receptor". The genetically modified cells will be purified and expanded to large numbers in the laboratory before infusion into the HIV-infected twin patient. The study is divided into two periods. In the first period, patients will receive single doses of CD8+ T cells with or without the "universal receptor". In the second period, patients will receive up to six doses of CD8+ T cells with or without the "universal receptor". This type of treatment is called adoptive immunotherapy. This collaborative clinical study between Cell Genesys and investigators at the NIH will evaluate the safety and tolerance of adoptive immunotherapy with CD8+ T cells containing the "universal receptor". By monitoring immune status, viral burden, organ function, and persistence of the cells in the body, we hope to determine whether this potential therapeutic approach is feasible and safe. This study may form the baseline for future protocols using T cells obtained directly from HIV patients.

"A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T Lymphocytes in HIV-Infected Identical Twins"

Recombinant DNA Advisory Committee Submission

Principal Investigator:	Robert E. Walker, M.D. LIR/NIAID
Associate Investigators:	R. Michael Blaese, M.D. DCBD/NCI Richard T. Davey, M.D.LIR/NIAID Judith Falloon, M.D. LIR/NIAID Craig Mullen, M.D. DCBD/NCI Michael A. Polis, M.D. LIR/NIAID
Study Sponsor:	National Institute of Allergy and Infectious Disease
Study Co-Sponsor:	Cell Genesys, Inc.
Date:	January 4, 1994

I agree to abide by all provisions set forth in the attached protocol.

_____ _____

Principal Investigator Date

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PROTOCOL SYNOPSIS

TITLE:	A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T-Lymphocytes in HIV-Infected Identical Twins
SPONSOR:	National Institute of Allergy and Infectious Diseases
CO-SPONSOR:	Cell Genesys Inc.
PHASE:	I/II
INDICATION:	HIV infection
STUDY DESIGN:	Open-label, comparative, sequentially randomized treatment with genetically unmodified or modified *ex vivo*-expanded T-lymphocytes in patients with HIV infection who possess a seronegative syngeneic twin. Genetic modification consists of introduction of a gene for HLA-unrestricted "universal" receptors specific for the gp120 HIV envelope protein. Treatment is divided into Periods I and II as outlined below under "Procedures".
PRIMARY ENDPOINT:	Safety of administering syngeneic genetically modified CD8+ cytotoxic T-lymphocytes.
SECONDARY ENDPOINTS:	Effects on primary disease status (CD4 count, HIV burden, p24 antigen), longevity of genetically modified transplanted T-lymphocytes.
SAMPLE SIZE:	See table below.
MAJOR ELIGIBILITY CRITERIA	Patients ≥18 years of age with HIV infection and a seronegative identical twin, Karnofsky ≥60%, life expectancy >6 months, adequate organ function,signed informed consent.
TEST ARTICLE:	Genetically modified, *ex vivo*-expanded syngeneic T lymphocytes.
COMPARISON:	*Ex vivo*-expanded, unmodified syngeneic T-lymphocytes.

Cohort	Treatment Period I		Treatment Period II
0	6 ->		6 + 4 new
1	6 ->		
2	6 ->	}	18 + 12 new at MTD
3	6 ->		

A maximum of 40 evaluable patients will be treated on this protocol: 10 given unmodified T-lymphocytes and 30 given modified T-lymphocytes.

DOSAGE AND ROUTE:

Cohort	Treatment Period I	Treatment Period II
0	10^{10} unmodified T-cells IV infusion x 1	Same x 6 q 8 weeks
1	10^{8} modified T-cells IV infusion x 1	10^{10} modified T-cells (or maximum safe dose) x 6 q 8 weeks
2	10^{9} modified T-cells IV infusion x 1	"
3	10^{10} modified T-cells IV infusion x 1	"

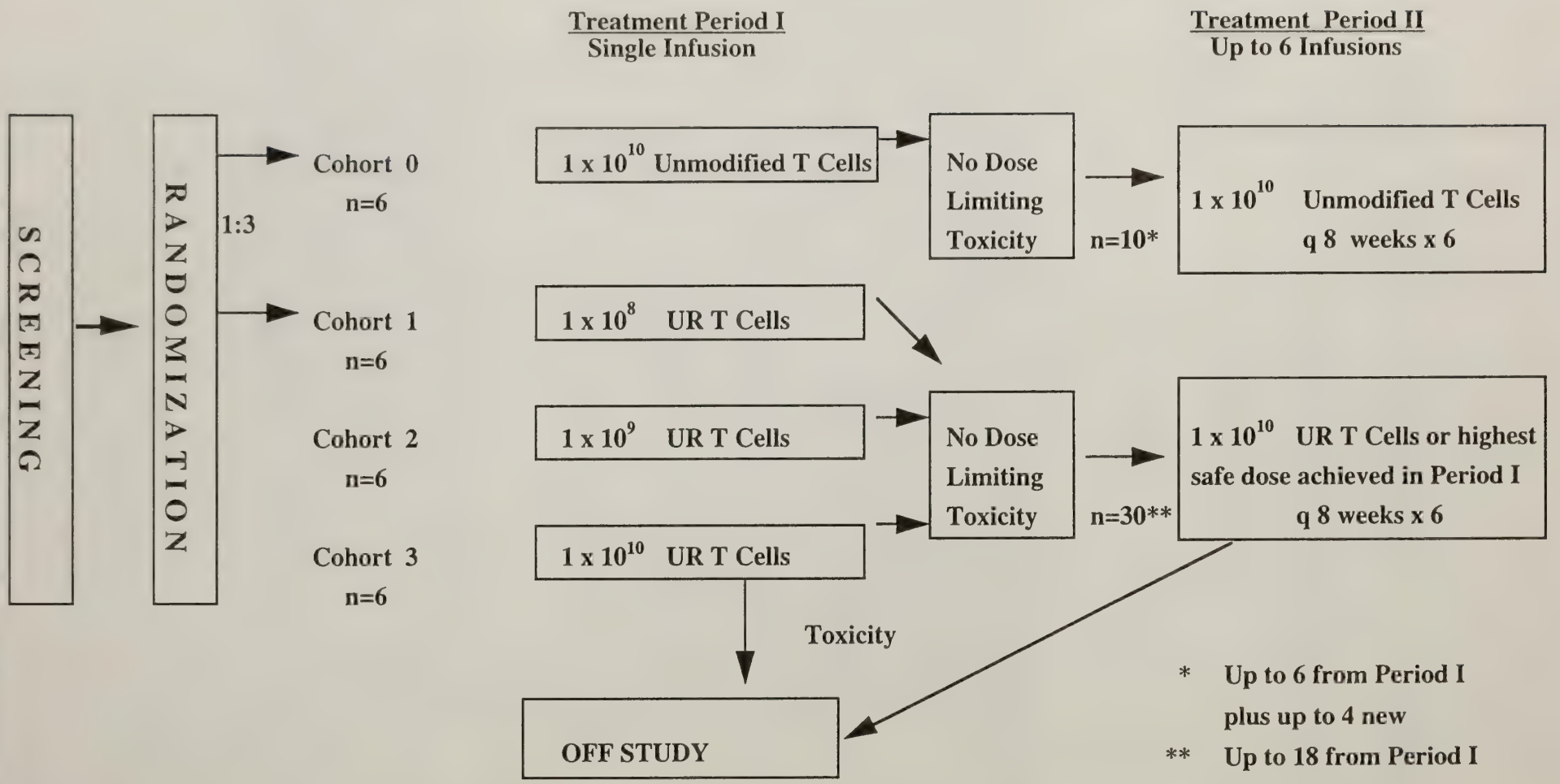
PROCEDURE:

T-lymphocytes from each seronegative twin will be obtained by lymphapheresis, separated into CD8+-enriched populations by monoclonal antibody binding techniques, activated by anti-CD3 and IL-2, and transduced using a retroviral vector. The introduced gene codes for a chimeric "universal receptor" which is HLA-unrestricted, recognizes the gp120 moiety of the HIV envelope, and is able to induce cytolytic activity against HIV-infected T-cells. The transduced T-cells then undergo primary expansion *ex vivo* 10- to 1,000-fold over approximately 8-10 weeks and are frozen in aliquots for later serial infusions. These transduced T-cells will then, in preparation for infusion into the respective seropositive twin, be thawed and will undergo a secondary expansion over 2-3 weeks.

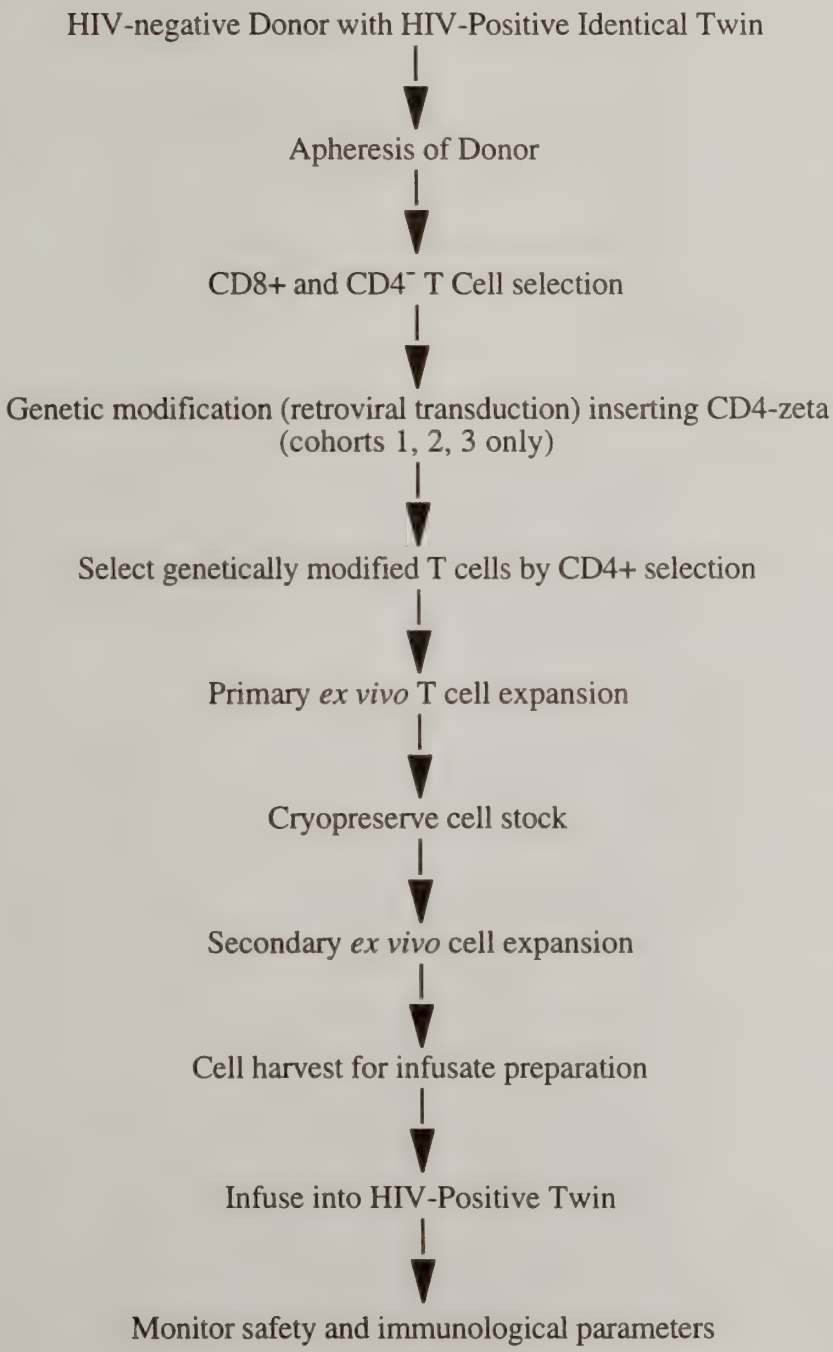
In Treatment Period I, patients are randomized in a 1:3 ratio to receive a single IV infusion of unmodified lymphocytes (Cohort 0 comparison) or genetically modified lymphocytes from their respective twins. Dosage levels of genetically modified lymphocytes will be filled sequentially, starting with 10^{8} cells (Cohort 1), 10^{9} cells (Cohort 2), and then 10^{10} cells (Cohort 3). Patients will be observed for safety variables; dose-limiting toxicity rates within each cohort will be assessed. All dosage cohorts will be filled before any patient advances into Period II.

In Treatment Period II, the 18 patients who in Period I received genetically modified lymphocytes without dose-limiting toxicity (cohorts 1, 2, 3) may be given up to 6 additional infusions at the maximum cell dose that was deemed safe in Period I. Up to 12 new patients may in addition be treated accordingly, to a maximum total of 30. The 6 patients who received unmodified lymphocytes in Period I (cohort 0) may receive up to 6 additional infusions during Period II at the same dosage; up to 4 new patients may be entered to a maximum of 10 per cohort. Safety, primary disease status, and standard immunological parameters will be assessed, including survival of the uniquely marked T-cell populations.

STUDY SCHEMA


Treatment Period I
Single Infusion
Treatment Period II
Up to 6 Infusions
SCREENING
RANDOMIZATION
1:3
Cohort 0
n=6
Cohort 1
n=6
Cohort 2
n=6
Cohort 3
n=6
1 x 10^10 Unmodified T Cells
1 x 10^8 UR T Cells
1 x 10^9 UR T Cells
1 x 10^10 UR T Cells
No Dose Limiting Toxicity
n=10*
No Dose Limiting Toxicity
n=30**
1 x 10^10 Unmodified T Cells q 8 weeks x 6
1 x 10^10 UR T Cells or highest safe dose achieved in Period I q 8 weeks x 6
Toxicity
OFF STUDY
* Up to 6 from Period I plus up to 4 new
** Up to 18 from Period I plus up to 12 new
UR = Universal Receptor

Protocol Flow Sheet


HIV-negative Donor with HIV-Positive Identical Twin
Apheresis of Donor
CD8+ and CD4- T Cell selection
Genetic modification (retroviral transduction) inserting CD4-zeta (cohorts 1, 2, 3 only)
Select genetically modified T cells by CD4+ selection
Primary ex vivo T cell expansion
Cryopreserve cell stock
Secondary ex vivo cell expansion
Cell harvest for infusate preparation
Infuse into HIV-Positive Twin
Monitor safety and immunological parameters

1. OBJECTIVES

1.1 Primary

1.1.1 To assess the safety and tolerance of the adoptive transfer of anti-HIV cytotoxic, syngeneic peripheral blood T-lymphocytes in patients with HIV infection.

1.2 Secondary

1.2.1 To determine the activity of adoptively transferred, genetically marked syngeneic peripheral blood anti-HIV cytotoxic T-lymphocytes in immune function of patients with HIV infection.

1.2.2 To determine longevity of the genetically marked CD8+ lymphocytes in the peripheral blood of patients with HIV infection.

2.1 Pre-clinical Studies

2.1.1 *In Vitro*

Numerous studies in rodents have demonstrated that the adoptive transfer of antigen specific CD8+ T cell clones can eradicate viral infection such as influenza (33,34). In addition, anti-HIV specific human T cell clones have been successfully generated (Protocol 92-1-0035). However, since these clones recognize antigen in an MHC-restricted fashion, they are not applicable as therapeutic agents for use as adoptively transferred cells in the majority of humans.

The adoptive immunotherapy-gene therapy approach utilized in this study redirects the antigenic-specificity of CD8+ cytotoxic T cells to kill HIV infected target cells using non-MHC restricted "universal" T cell receptors (URs). Upon binding to the viral antigen, these anti-HIV specific URs initiate T cell activation, resulting in induction of effector functions, including cytolysis of the HIV-infected cell. In these chimeric receptors, the cytoplasmic domain of zeta is fused to an extracellular domain of the targeting molecule, which confers specificity for HIV env protein. The UR derives its specificity from the extracellular domain of the human CD4 receptor which recognizes the gp120 moiety of HIV env, currently the only well characterized high affinity receptor for HIV.

Expression of the CD4-UR in a model T cell line enabled the cells to initiate T cell effector functions such as cytokine secretion in response to HIV-env expressing target cells. These initial experiments served to demonstrate functionality of the anti-HIV URs in T cell activation. In the subsequent experiments, their ability to mediate T cell effector functions in primary human CD8+ T cells was evaluated. We therefore introduced the UR via retroviral transduction into human CD8+ T cells purified by positive selection from the peripheral blood mononuclear cells (PBMCs) of normal donors. Purified populations of CD8+ T cells were subsequently isolated that expressed surface UR as seen by fluorescence analysis, and have integrated provirus in the genome as demonstrated by Southern analysis. The HIV-specific UR was stably expressed at high levels on the surface of transduced CD8+ T cells modified in this manner. The structure and

expression levels of the UR were functional in T cell activation as defined by their ability to initiate proliferation and cytolytic activity in response to target cells expressing very low levels of HIV antigen. Importantly, the CD8+ UR+ T cell population exhibits highly efficient cytolytic activity against CD4+ T cells infected with HIV in an antigen specific MHC-unrestricted manner.

The CD4-UR initiate levels of cytolytic activity against 293env target cells, exhibiting specific lysis at E:T ratios as low as 0.3:1. Subsequent studies were designed to evaluate the effectiveness with which the CD8+ UR+ T cell populations were able to mount a cytolytic response against target cell types more closely resembling those encountered in an HIV-infected individual, namely HIV-infected CD4+ T cells. The CD4+ T cell line CEM is readily infectable by the laboratory viral isolate HIV IIIB. When the CEM.IIIB cell population were used as targets in a cytotoxicity assay, the results obtained were even more dramatic than had been observed with the 293env target cells: E:T ratios as low as 0.075:1 resulted in specific lysis of the virally infected population, maximal lysis occurring at E:T ratios as low as 2.5 -5.0:1 with levels of specific lysis approximately 90% above background. The explanation for the increased activity of the UR+ CD8+ T cells against CEM.IIIB compared to 293env, may simply reflect the dramatic difference in surface antigen levels observed between the two target cells, but also may involve cell-specific factors differing between the target CEM T cells and 293 epithelial cells.
In addition, CD8+ UR+ T cell populations were evaluated for their ability to proliferate in response to 293 target cells expressing HIV env. Redirected UR+ T cells were incubated with mitomycin C-treated 293.env or control 293.neo cells for 3 days, and 3H-Thymidine incorporation was measured. In parallel, the CD8+ UR+ T cell population was stimulated with the CD3-specific antibody OKT3 in order compare the proliferative response to that obtained by highly efficient stimulation of the native T cell receptor by antibody cross-linking. The CD4-UR+ cells proliferate extensively in response to stimulation with gp120 or gp41 respectively expressed on the surface of 293env cells. The proliferative response of the modified T cell population to stimulation through the UR with surface viral antigen is comparable to that obtained by cross-linking the native T cell receptor with specific antibody.

The experimental design outlined here is directly applicable to the clinical setting, involving purification of PBMC-derived CD8+ T cells from the blood of human donors, introduction of the UR gene via retroviral transduction, purification of transduced UR+ T cells, expansion, and reinfusion into the patient. The transduced T cell populations produced in this manner express high levels of anti-HIV UR, and are able to mount highly efficient effector T cell responses such as cytokine secretion, proliferation, and cytolytic activity upon interaction with target cells expressing extremely low levels of surface HIV antigen. This approach can be used to obtain large populations of redirected T cells in a matter of weeks.

The relative affinity of the CD4 receptor for nonpolymorphic sites on HLA class II molecules is thought to be very low in absence of additional stabilizing TCR-HLA interactions. However, the potential for CD4-UR modified T cell populations generated to initiate a cytolytic response against

uninfected cells expressing HLA class II was evaluated. Cytotoxicity assays employing a cell line that expresses very high levels of HLA class II as a target, namely the human B cell line Raji, demonstrated that CD4-UR+CD8+ T cells were unable to kill this HLA class II-expressing target, even at effector: target ratios as high as 20:1.

Since the genetically modified CD8+ lymphocytes will express the CD4-UR cell-surface protein, the theoretical possibility exists that these transduced cells will become susceptible to HIV infection, as the virus gains entry into cells using the CD4 glycoprotein as a receptor. Consequently, studies were designed to test the infectability of CD4-UR+CD8+ T cells in vitro. Using two different strains of virus (HIV-1 IIIB and JR-CSF) in 7-day cultures, preliminary results have detected no evidence of infection in the genetically modified cells based on absence of p24gag in the supernatant (data on file, Cell Genesys, Inc.).

2.1.2 Assessment of the Safety of Retroviral-mediated Gene Transfer

In the ADA-deficient SCID clinical protocol, a total of approximately 20 infusions of up to 2 x 10^{10} cultured cells have been performed, and no significant side effects related to the cell infusions have been observed. In addition, there have been no side effects related to gene transfer. Similarly, in the N2/TIL protocol, there have been no side effects due to gene transfer and no evidence of infection by replication-competent virus. Since foreign DNA is inserted randomly into the genome of cells that are reinfused into the patient, however, there is some potential for the insertional event to result in an unfavorable outcome. If the insertion disrupts a gene essential for maintaining cell function, that particular cell might die. More threatening is the possibility that insertion may initiate oncogenic transformation of the cell. The magnitude of the risk that gene transfer poses to a patient cannot be accurately stated at present, but based on the accumulated experience the risk appears to be very low.

Some of the potential hazards were illustrated in a primate model of retrovirally-mediated gene modification, in which lethally irradiated rhesus monkeys were exposed to massive amounts of replication-competent retrovirus. When autologous stem cells used to reconstitute the bone marrow were incubated in vitro in the presence of retroviral vector particles with accompanying replication-competent virus, retroviral vector genome was detected in circulating cells of 5 of 8 of the transplant recipients of CD34+ cells and in 2 recipients of unfractionated bone marrow cells (35). Three recipients of CD34 cells had a productive infection with replication-competent virus. Within 7 months of transplantation, each of these three animals developed a rapidly progressive T cell neoplasm; lymphoma cells contained 10 to 50 copies of the replication-competent virus but, importantly, lacked the retroviral vector. Thus, these tumors occurred in the setting of intentional contamination of the bone marrow transplant with replication-competent virus, were associated with continuous retroviremia, but did not involve the retroviral vector. This experience underscores the importance of carefully screening retroviral producer clones used in human trials to exclude contamination with replication-competent virus.
In the more than 30 patients who have now received gene-modified cells, no evidence for the generation of replication-competent virus has been

seen. Long term surveillance of exposed monkeys as well as the patients enrolled in the various clinical protocols will be necessary to better understand the actual frequency and severity of the risks associated with this procedure.

2.2 Clinical Experience

2.2.1 In protocol 92-I-248, 12 HIV seropositive identical twins have received between 1 and 4 infusions of activated, expanded, unfractionated lymphocytes from their seronegative twin. The infusions have generally been well-tolerated. Some of the infusions have been associated with fevers, chills, headaches, myalgias, fatigue, and/or hypotension requiring intravenous fluids within 24-48 hours, currently thought to represent a type III hypersensitivity reaction to bovine proteins in previously sensitized individuals, as the cells are cultured in media supplemented with fetal calf serum. Transient increases in CD4 counts have appeared in most recipients within 2 weeks post-transfer. These increases have generally waned during the subsequent few weeks prior to the next cell transfer. Several recipients have demonstrated evidence of viral activation immediately post-transfer, but this has not been universal.

2.2.2 *In Vivo* Human Experiments in SCID with Autologous PBLs Transduced with the ADA Gene

Two patients with severe combined immunodeficiency due to adenosine deaminase deficiency have been treated with infusions of autologous lymphocytes transduced with the ADA gene via a retroviral vector (protocol #90-C-195). To date, no significant side effects have occurred and immunologic benefit has been observed. Specifically, reversal of lymphopenia, defective T cell cytolytic activity, skin test anergy, and deficient antibody production have been achieved in these patients associated with gene/T cell therapy. Continuous circulation of modified cells during 21 months of observation has been seen in the first patient enrolled on the protocol, including during a 7 month period when this patient received no additional infusions of cells. ADA levels in the circulating T cells have increased from initially undetectable levels to 10% to 50% of that measured in the carrier parents.

The studies of these children with SCID demonstrate that significant reconstitution of T cell function can be achieved in immunodeficient patients with infusions of autologous polyclonal T cells activated for short term (<2 weeks) growth in culture.

2.2.3 *In Vivo* Human Experiments with TIL Transduced with the LNL6 Vector

The human gene transfer N2/TIL clinical protocol (86-C-183c) was approved in 1989. In this study, 10 patients with advanced malignant melanoma received tumor infiltrating lymphocytes (TIL) that have been marked with the safety-modified N2 vector called LNL6. Extensive safety studies on the retroviral supernatant prior to transduction of human TIL, the transduced TIL before infusion into the patient, and the patients who received the gene marked TIL were performed.

In summary of the safety data, there have been no side effects or toxic reactions due to the gene transfer. In addition, there has been no evidence of replication-competent virus in any retroviral supernatant used for clinical studies, no consistent differences in the pattern of cell growth, phenotype, or cytotoxic function in any preparation of gene-transduced TIL, and no lymphocyte population has developed IL-2 independence. There has also been no evidence of viral exposure for any of the 10 patients based on Western analysis as well as 3T3 amplification with S+L- cell assay of patient serum. In short, the data acquired to date demonstrate no abnormalities, side effects, toxicity's, or pathology due to the retroviral-mediated gene transfer procedure.

2.3 Rationale for the Current Study

The proposed protocol is designed to evaluate the safety and potential value of genetically modifying T lymphocytes in an attempt to prevent or control HIV infection. A large amount of experimental data have accumulated which suggest that the CD8+ T cell response may represent the major and earliest immune response to HIV infection. For example, studies have shown that anti-HIV CTL are present in the PBMC of HIV sero-negative individuals exposed to HIV (36). Although a high frequency of HIV antigen-specific CD8+ T cells are also found in the peripheral blood of asymptomatic HIV seropositive patients, a selective decline in HIV specific CTLs is observed in patients as they progress to later stages of the disease, even though polyclonal cytolytic activity in these patients appears to be unaffected (37). This suggests that a breakdown of the host cell-mediated immune response is associated with progression to AIDS. In vitro studies have not only shown that anti-HIV CD8+ cells exhibit cytolytic activity toward HIV infected targets, but also have demonstrated that these cells have the ability to inhibit HIV replication in lymphocyte cultures (38). These results suggest that adoptive transfer of HIV specific CTLs may have potential as immunotherapy for HIV infected individuals. In order to generate large numbers of anti-HIV specific CTL's suitable for infusion into AIDS patients with various HLA types, a method has been developed using retroviral mediated gene transfer to insert a HIV-targeting gene into primary CD8+ T cells. The genetically marked cells are then purified and expanded, and therefore large numbers of HIV specific CTL's can be generated for infusion. From rodent adoptive immunotherapy studies, it has been determined that anti-viral effects can be demonstrated; however, large numbers of specific anti-viral CTL's need to be infused.

This study will provide data on the effect of infusion of specific anti-HIV cytotoxic CD8+ T cells into HIV infected individuals. In addition, the present study will permit the elucidation of effects of infusion of specific anti-HIV effector cells as opposed to infusion of bulk populations of polyclonal CD8+ T cells. By monitoring functional immune status, viral burden, and end organ function, we hope to determine whether this potential therapeutic approach is feasible and safe. This study will form the baseline to which other protocols using lymphocytes directly from HIV patients will be compared.

3. EXPERIMENTAL PLAN

3.1 Study Design

- Phase I / II
- Patient Population - HIV-infected patients with an uninfected syngeneic twin
- Randomized

- Number of Treatment Groups - Three sequential dosage groups, one comparative group
- Comparative Group - Non-modified syngeneic lymphocytes
- Primary Endpoint - Safety
- Secondary Endpoints - Effects on primary disease status (CD4 count, HIV burden, p24 antigen), longevity of genetically modified cells.

3.2 Number of Institutions

- One institution is expected to participate in this trial.

3.3 Number of Patients

- Required Evaluable per dosage cohort in Treatment Period I - 6
- Total Number of Evaluable Patients - 40 maximum

3.4 Estimated Study Duration

- Treatment Duration for Individual Patient - Approximately 1 year
- Total Study Duration - Approximately 2 years

4. PATIENT ELIGIBILITY

Screening tests for eligibility must be completed within 2 weeks of a patient's being registered onto the study. If more than 2 weeks elapse between screening and apheresis of the donor, HIV positivity tests for the donor must be repeated.

4.1 Inclusion Criteria

4.1.1 An identical twin pair, one of whom is seropositive for HIV, the other twin seronegative, by standard ELISA, PCR, and Western blot testing.

4.1.2 Patients with Kaposi's sarcoma are eligible for this study, but must not have received any systemic therapy for KS within 4 weeks prior to entry. The diagnosis of KS must have been confirmed by biopsy.

4.1.3 Anticipated survival > 6 months and Karnofsky Performance Status $\geq$ 60% (Appendix A).

4.1.4 18 years of age or older.

4.1.5 Free from serious psychological or emotional illness and able to provide written informed consent.

4.2 Exclusion Criteria - Recipient

4.2.1 Lymphoma

4.2.2 Unwillingness to comply with current NIH Clinical Center guidelines concerning appropriate notification of all current sexual partners of an individual regarding his or her HIV positive sero-status and the risk of transmission of HIV infection.

4.2.3 Recent history of substance abuse unless evidence is provided of an ongoing therapeutic intervention (i.e. medical therapy or counseling) to control such abuse.

4.2.4 Pregnancy

4.3 Exclusion Criteria - Donor

4.3.1 Untreated or inadequately treated medical condition (e.g., cardiopulmonary disease, acute infection) which, in the judgment of the Principal Investigator, precludes apheresis.

4.3.2 Serologic positivity for either Epstein Barr virus or Cytomegalovirus if and only if the recipient twin tests seronegative for the corresponding virus.

5. LYMPHOCYTE PROCESSING

The anti-HIV targeting gene introduced into CD8+ lymphocytes was generated by recombinant DNA technology and will produce a chimeric protein consisting of the extracellular portion of CD4 fused to the zeta subunit of the T cell receptor. The gene is packaged into a retroviral particle as a means for efficient delivery into lymphocytes.

The retroviral particles are produced by Cell Genesys, Inc. Cells generating the recombinant retroviral particle are grown in culture under defined and controlled conditions. The supernatants are filtered and frozen. The CD8+ lymphocytes derived from the donor twin will be transduced and expanded by Biotechnology Research Institute (BRI) as outlined below.

5.1 Isolation and Enrichment of Peripheral Blood Lymphocytes From Donor

Lymphapheresis (performed by the NIH Blood Bank Apheresis Unit according to standard procedures) will be used to obtain lymphocytes from the donor using an automated cell separator. Apheresis will commence at 8:00 A.M. so that processed cells may reach BRI before 1:00 P.M. that same day. Lymphocytes will be harvested and the remaining red cells and plasma returned to the donor as follows: Whole blood is withdrawn from one venipuncture site at a rate of 40-60 ml/min and channeled into the cell separator, where cellular and plasma fractions are separated by centrifugation. The lymphocytes are harvested into a component bag, and the red cells and plasma are re-infused into the donor via a second venipuncture site. Anticoagulation is achieved using ACD-A at a whole blood to anticoagulant ratio of 10:1. Maximum extracorporeal blood volume is 300 ml (Fenwal, COBE models). Two to four hours are required to process 10 liters of whole blood.

A minimum of 5×10^9 total peripheral blood mononuclear cells (PBMC) is required. The pheresis procedure should be continued until the requisite yield is obtained.

5.2 Shipping Procedure to Biotechnology Research Institute

The lymphopacks from each apheresis will be transported to Biotechnology Research Institute as outlined in Appendix B.

5.3 Cell Separation and Enrichment of CD8 T Cell Sub-populations

Peripheral blood mononuclear cells from the lymphopacks will be fractionated into populations enriched for CD8-expressing cells. CD8 enrichment will be achieved by positive selection using an immunoaffinity-based system with a CD8-specific monoclonal antibody. CD4 depletion will be performed and the resulting

subpopulations will be termed "CD8-enriched cells". Enrichment will be performed prior to transduction of the cellular fractions.

A minimum of 1.5 x 10^8 "CD8-enriched cells" prior to transduction is required. In the event of insufficient yield, the donor may be required to return for a second apheresis. Non-availability of the donor or a second yield failure may be cause for removal of the twin pair from study.

5.4 Growth and Transduction of T Lymphocytes

Randomization will take place at this stage, which will determine whether or not the CD8+ enriched cells from a particular donor will be transduced. In each case, half of the "CD8-enriched cell" fraction will be frozen and saved as backup (at least 5 x 10^7 cells).

The other half of the fractionated cells will be cultured at approximately 5 x 10^5 cells/well in 24-well tissue culture plates in 100 units/ml of IL-2 (Chiron). Media may be supplemented with human AB serum. At the initial plating, 10 ng/ml OKT3 (Ortho) monoclonal antibody will be added to each well. The cells will be cultured at 37° C in a humidified incubator with 5% CO_2.

Once the T lymphocytes from the donor of the patients randomized to cohorts 1, 2, and 3 have begun to proliferate (usually 3-4 days after initiation of the culture), vector-containing supernatant will be added to the wells after aspirating off the top half of the medium. This will be repeated 5 times over 2-3 days. After the final exposure to retroviral vector, the genetically altered cells will be purified by immuno-affinity techniques (via CD4 receptor). After purification, the cells will be fed with fresh media and cultured another 2 to 7 days to permit the cultures to return to exponential growth.

The percentage of transduced cells will be determined by co-expression of the CD8+CD4+ phenotype. The required transduction frequency is >1% *and* with a minimum of 1 x 10^6 transduced cells after CD4+ selection. If this yield was not obtained from the initial transduction attempt or using the backup cells, either the donor will be repheresed and the cell processing repeated or the twin pair will be considered off-study.

Once cell volume reaches approximately 400 ml (4 x 10^8 cells), the cell cultures from all donors will be expanded up to 2 x 10^{10} cells employing specialized Life-CellTM Tissue Culture flasks which are designed similarly to blood unit bags but are gas-permeable and hermetically sealable. It is estimated that the primary expansion will require 8-10 weeks. During the expansion steps, it may be necessary to restimulate the cells with OKT3 and irradiated syngeneic PBMC. Ten aliquots each containing approximately 1.5 x 10^9 cells will be cryopreserved in order to provide adequate stock cell cultures for subsequent cell expansions for Treatment Period I and II. Prior to freezing, an aliquot will be taken for replication-competent retrovirus (RCR) testing. Results should be available prior to infusion of cells into the seropositive recipient.

All cell expansions will be performed with strict adherence to aseptic technique. Cell cultures will be tested for sterility employing both aerobic and anaerobic microbacteriological procedures. A small sample of expanded T-cells will be analyzed by standard flow cytometric phenotypic analysis for CD3, CD4, and CD8 expression.

Cytolytic function of transduced and *ex vivo* expanded T-cells will be determined by microlymphocytotoxicity assays employing 293 cells (human embryonic kidney line) expressing gp120 as cytolytic targets. Tests for sterility, phenotype, function, and percent of transduced cells will be performed by BRI.

5.5 Preparation of Cells for Infusion

Approximately 2-3 weeks prior to the expected day of cell infusion, aliquots of stocked, cryopreserved cell cultures will be thawed and grown in a secondary expansion step to the required dose in accordance with treatment assignment (aiming for >1 x 10^{10} cells) in humidified incubators under 5% CO_2 at 37°C. Forty-eight hours prior to each scheduled harvest of the expanded cell cultures for subsequent infusion, a representative number of expansion bags will be tested for sterility as before. Once cell cultures attain the required number, cells will be aseptically harvested and pooled for infusion using a specialized cell harvester (Baxter or Dupont) and a Baxter Life-Cell™ Tissue Culture gas-permeable bag of a 1 liter capacity. Cells are then filtered through a 170 micron filter in a solution of physiologic saline containing 2% human albumin, in preparation for infusion into the patient. Standard tests for sterility and percentage of transduced cells will be performed. Infusion will proceed if the proportion of CD8+CD4+ phenotype exceeds 70% of the expanded cells.

Units of expanded cells ready for infusion will be transported to the NIH Clinical Center by the BRI courier, under the conditions outlined in Appendix B.

6. TREATMENT PROCEDURES

6.1 Treatment Period I

Treatment groups are shown in the table below. For cohort 0, lymphocytes pheresed from the seronegative twins will be fractionated into CD8+ cells and expanded *ex vivo*, but without genetic modification. The corresponding seropositive twins will receive a single infusion at a cell dose of 1 x 10^{10}. For cohorts 1-3, the seronegative twin will likewise undergo apheresis, and the lymphocytes will be fractionated into CD8+ cells, activated, transduced with a retrovirus carrying the gene for the chimeric protein targeting HIV-infected cells, and expanded *ex vivo*. The initial 6 corresponding seropositive twins will receive a lymphocyte infusion of 1 x 10^8 cells (cohort 1). If at the first dose level no immediate dose-limiting toxicity has manifested in the minimum numbers of patients outlined in Section 11.1.3, randomization will proceed to cohort 2 (1 x 10^9 cells). The same rules of advancement will be followed for cohort 3 (1 x 10^{10}). Following the single infusion of cells, patients will be observed through 8 weeks for safety.

Cohort	Lymphocyte Dose	Planned Number of IV infusions	Minimum Number of Patients
0	1 x 10^{10} unmodified T-cells	1	6
1	1 x 10^8 UR T-cells	1	6
2	1 x 10^9 UR T-cells	1	6
3	1 x 10^{10} UR T-cells	1	6

HIV-infected patients will be admitted to the Warren G. Magnuson Clinical Center inpatient wards for the first single infusion in Period I. An intravenous catheter will be

inserted into either a peripheral vein or, if no peripheral access is available, into a central vein. In accordance with the randomization, either 1 x 10^8, 1 x 10^9, or 1 x 10^{10} expanded lymphocytes will be infused over 60 minutes. Patients will be under close inpatient observation for a total of 4 hours from the start of the infusion, and will be seen as an outpatient over the next 3 days.

Vital signs will be taken pre-infusion, every 15 minutes during the infusion, then hourly for the remainder of the 4-hour observation period. Oxygen saturation by pulse oximetry will be taken continuously during the infusion and 4-hour observation period. Patients who develop fever, chills, or muscle aches may receive aspirin, ibuprofen, or acetaminophen as indicated. Acetaminophen will be used if the patient does not tolerate aspirin or ibuprofen. The development of a rash will be treated initially with diphenhydramine PO. Cell infusions will be slowed or discontinued depending upon the severity of the reaction. In the event of a severe reaction, emergency life support measures will be immediately undertaken. Patients who experienced reactions to a previous infusion may be premedicated 30 minutes prior to future infusions with either aspirin, acetaminophen, or diphenhydramine. Meperidine will be used for more severe chills and muscle aches that do not quickly respond to antipyretics and antihistamines.

Patients experiencing rapid disease progression or dose-limiting toxicity to the lymphocyte infusions (as defined in Section 11.1) during Treatment Period I will terminate participation but will go through safety observation. These patients will not participate in Treatment Period II. Every effort will be made to retain patients on-study as long as treatment is not felt to be contraindicated. These dropouts will not be replaced.

Criteria for the advancement of dose escalation and definitions of dose-limiting toxicity, are outlined in Section 11.1. In the event of a marginal toxicity rate at any one dosage level, three additional patients may be enrolled in order to further define the trend as outlined in Section 11.1.4.

No patient will proceed into Treatment Period II until patients in all 3 cohorts have been assessed for safety. Therefore, there will be a variable period of delay between Periods I and II during which the patients will still be considered on-study and on whom follow-up will be maintained.

6.2 Treatment Period II

After the requisite number of patients in all treatment cohorts have received their single Period I infusion of cells and each cohort assessed for the rate of immediate dose-limiting toxicity (DLT), the seropositive twins who did not exhibit DLT may continue into Treatment Period II after at least 8 weeks of observation.

Patients will be reassessed for eligibility *vis a vis* the criteria outlined in Section 4; those who are no longer eligible will terminate the study at that time. Patients originally randomized to cohort 0 will receive up to 6 additional infusions of unmodified lymphocytes at the same dose of 1 x 10^{10} cells, given every 8 weeks. Patients in cohorts 1, 2, and 3 will all receive the highest cell dose that was deemed safe in Period I according to the rules in Section 11.1. Up to 16 additional patients may be newly randomized to receive either 10^{10} unmodified lymphocytes (4 patients) or modified lymphocytes at the highest dosage deemed safe in Period I (12 patients).

Subsequent infusions in Period II may be performed on an outpatient basis at the discretion of the Principal Investigator. For outpatient infusions, patients will be

treated in the 8th floor clinic of the Clinical Center or in the 11th floor day hospital. Each infusion will be administered over 1 hour and the patient will be observed for a total of 4 hours. As before, vital signs will be taken pre-infusion and every 15 minutes during the infusion, then hourly through 4 hours. Patients will return to the Clinical Center for outpatient evaluation daily for 4 days after each infusion, then weekly or less frequently (to be guided by the data produced) for the duration of active treatment, then monthly for 6 months of follow-up beyond the last lymphocyte infusion. Comprehensive safety and immunologic evaluations will be performed according to Section 8.

6.3 Ancillary Therapy

Licensed antiretroviral drugs, antiretrovial drugs available through an FDA approved expanded access program, and licensed drugs for HIV-related conditions are permitted on this protocol. Patients may continue to receive these medications, switch to different doses, and switch to different drugs throughout the course of the present study. Other experimental drugs are not permitted while on this study. All prescription and non-prescription concomitant medications will be recorded, listing generic name (unless a combination formulation), indication, dosage, route, and inclusive dates of administration.

7. TESTS AND OBSERVATIONS

7. 1 Screening

Both donor and recipient will undergo the below screening tests. If more than 2 weeks elapse between screening and apheresis, the donor will be retested for HIV seronegativity.

- Medical history (see Appendix C);
- Complete disease characterization (see Appendix C);
- Complete physical exam and Karnofsky Performance Status;
- Chest x-ray and EKG;
- Urine pregnancy test for females;
- Laboratory tests (see also Appendix C).

    - Hematology and coagulation tests;
    - Blood chemistries and electrolytes;
    - Urinalysis including microscopic;
    - Blood samples for RCR testing.

7.2 On-Treatment

The following tests will be performed for the seropositive twin within each stage of the study at the indicated intervals:

Treatment Period I

- Complete physical examination (as before) day 1, and at week 5 post infusion;
- Vital signs (pulse, respiration, supine blood pressure, oral temperature) at times 0, 15, 30, 45, and 60 minutes during infusion, then hourly during the 4-hour observation period;
- Oxygen saturation by continuous pulse oximetry throughout the infusion and 4-hour observation;

- Hematology and coagulation (as before) at baseline and every 2 weeks thereafter (weeks 3, 5, 7);
- Blood chemistries (as before) at baseline and every 2 weeks thereafter (weeks 3, 5, 7);
- Blood sample for special laboratory studies:
  - CD4, acid-dissociated p24 antigen, viral burden (e.g., branched DNA or bDNA) days 1-4, then every 2 weeks thereafter (weeks 1,3,5,7);
  - PCR for CD4-zeta gene days 1-4 and every 2 weeks thereafter or until no detectable signal on 2 consecutive samples;
  - RCR testing monthly (e.g., weeks 5 and 9);
- Urinalysis at baseline and every 2 weeks thereafter (weeks 3, 5, 7);
- Continuous record of adverse events (see Sections 10 and Appendix D).

Treatment Period II

- Vital signs at times 0, 15, 30, and 60 minutes during the infusion on day 1;
- Oxygen saturation by continuous pulse oximetry throughout the infusion period;
- Complete physical examination (as before) at start of period (week 1), and at week 5 of each 8-week treatment);
- Hematology and coagulation (as before) monthly (weeks 1 and 5) of each 8-week treatment);
- Blood chemistries (as before) monthly (weeks 1 and 5) of each 8-week treatment);
- Blood sample for special laboratory studies:
  - CD4, acid-dissociated p24 antigen, viral burden (e.g., bDNA) days 1-4 then at week 5 of each 8-week treatment;
  - PCR for CD4-zeta gene days 1-4 and every 2 weeks or until no detectable signal on 2 consecutive samples;
  - RCR testing monthly (weeks 1 and 5);
- Urinalysis monthly (weeks 1 and 5);
- Continuous record of adverse events until resolution unless deemed non-drug related or irreversible.

7.3 Off-Study

When the patient completes the study or terminates early for any reason, the following should he performed if at all possible:

- Complete physical examination and performance status;
- Disease evaluation;
- Final laboratories (hematology and coagulation, blood chemistries, RCR testing, urinalysis);
- Status of adverse events;
- Reason for study removal.

7.4 Follow-up

The Investigator will assess the patient after the last treatment period for the following:

- Treatment and monitoring of all adverse events until resolution, unless disease-related, non-drug related, or irreversible;
- RCR testing (as before) monthly for 3 months after study completion, quarterly for one year, then annually thereafter;
- Performance and disease status monthly for 6 months.

8. STATISTICAL CONSIDERATIONS

8.1 Definitions

8.1.1 Evaluable for Analysis

All patients who receive at least one lymphocyte infusion will be analyzed for safety (with respect to AEs) and where possible immunological activity. Patients who discontinue study prematurely will not be replaced but all due effort will be made to follow them for outcome variables. These patients will be censored from the analysis at the point at which their data are no longer available.

8.1.2 Dose-Limiting Toxicity (DLT)

Any of the following adverse events which are considered probably or definitely related to genetically modified lymphocyte transfusions:

- Grade 3 or greater adverse event (see Appendix D);
- Unacceptable grade 2 adverse event in the opinion of the Principal Investigator;
- Other severe or life-threatening complication or abnormality.

8.1.3 Maximum Tolerable Dose (MTD)

The maximum dose tested at which 33% or fewer patients experience DLT.

8.1.4 Criterion for Dose Advancement

All patients within a dose cohort must complete a single infusion of lymphocytes and be evaluable for immediate dose-limiting toxicity. Either less than 2 of 6 patients or less than 4 of 9 patients may experience a dose-limiting toxicity in order to advance to the next dose level.

Specific criteria for advancement to the next higher dose level are as follows:

DLT in:	Then:	DLT in:	Then:
0 or 1 of 6 Pts ->	Start next dose level		
2 of 6 Pts ->	Add 3 Pts ->	0 or 1 of 3 ->	Start next dose level
		> 2 of 3 ->	Stop enrollment at this dose; previous level is MTD
> 3 of 6 Pts ->	Stop enrollment at this dose; do not start next dose level		

All patients who had been randomized into cohorts 1, 2, and 3 for Period I

will be treated at the MTD during Period II (or at 1 x 10^{10} cells if no MTD was described).

8.1.5 Eligibility for Treatment Period II

Patients who proceed successfully through Treatment Period I in the absence of dose-limiting toxicity as defined above will be considered for Treatment Period II. Patients must still fulfill entry criteria as outlined in Section 4.

8.2 Study Design

This is a sequential, open-label, randomized, controlled, Phase I / II clinical trial to study the safety of 3 different doses of genetically modified T lymphocytes in the setting of patients with HIV infection. The study will be conducted at one center. In addition, the activity of genetically modified lymphocyte infusions, as determined by effect on immunological status, will be descriptively assessed.

The study has two distinct Treatment Periods. During Treatment Period I, HIV positive patients presenting at the participating center will be randomized in a 1:3 ratio to receive unmodified lymphocytes (cohort 0 comparison) or genetically modified lymphocytes at 1 of 3 sequential dosage levels (cohorts 1-3). Cohort 1 will be filled and rate of dose-limiting toxicity assessed prior to cohort 2 being initiated, and the same for Cohort 3. A minimum of 6 patients at each dose in the genetically modified group will be enrolled in Treatment Period I. Evaluation of the safety of each dose of lymphocyte infusions will be determined by assessing the aggregate rates of adverse events. Advancement to the next dosage cohort will occur only if the required numbers of patients do not experience a DLT at the current dose level (see 11.1.4).

Safety of the three dose levels of lymphocytes will be determined by comparing the adverse event (AE) rates and changes in vital signs and laboratory values in the three groups to patients receiving unmodified, *ex vivo* expanded syngeneic lymphocytes, and to baseline patient status prior to any cell infusions.

Indices of the primary disease, HIV infection, will be followed for all patients, both as a safety endpoint (to monitor untoward acceleration of disease) and an activity endpoint (ability of the transfused lymphocytes to ameliorate viral burden). These variables include change in CD4 count, change in p24 antigen, and change in HIV viremia.

8.3 Sample Size Determination

8.3.1 Treatment Period I

Six or nine patients evaluable for safety will be studied in the dose escalation phase (Treatment Period I). The following table lists the probability of observing various unacceptable toxicities as a function of the true rate of occurrence in the population of HIV infected patients treated as per protocol (based on binomial probabilities):

# cases of DLT Seen (n=6)	True Rate of Occurrence in Population				
	10%	20%	30%	40%	50%
0 or 1+	0.885	0.655	0.421	0.235	0.100
2++	0.098	0.246	0.324	0.311	0.234
>2+++	0.016	0.099	0.256	0.455	0.656

\+ Outcomes Leading to Escalation to Next Dose: Probability of (0/6) or (1/6)
++ Outcomes Leading to Adding 3 Patients: Probability of (2/6)
+++ Outcomes Leading to Stopping: Probability of (3/6) or (4/6) or (5/6) or (6/6)

-OR-

# cases of DLT Seen (n=9)	True Rate of Occurrence in Population				
	10%	20%	30%	40%	50%
0 or 1	n/a	n/a	n/a	n/a	n/a
2 or 3*	0.095	0.220	0.254	0.202	0.117
4 or 5**	0.003	0.026	0.070	0.109	0.117

* Outcomes Leading to Escalation to Next Dose: Probability of [(2/6) and (0/3)] or [(2/6) and (1/3)]
** Outcomes Leading to Stopping: Probability of [(2/6) and (2/3)] or [(2/6) and (3/3)]

With a sample size of 6 or 9 patients per dose cohort, it would be very likely (i.e. 77% chance) to see 33% (or more) of patients who experience a DLT if the true rate of occurrence was >50%. There will be less than a 12.5% chance of calling toxicities dose limiting if the true rate of occurrence is 20% or less.

8.3.2 Combined data: Treatment Periods I and II

Exploratory analyses will be conducted using a dichotomous categorizations of immunologic or virologic improvement based on CD4+, p24, and/or plasma viremia. With 30 patients in the genetically modified lymphocyte group, the proportion of patients experiencing immunologic or virologic improvement can be estimated with a standard deviation less than 0.10.

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Informed Consent

MEDICAL RECORD — CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY
° Adult Patient or °Parent, for Minor Patient

INSTITUTE: NIAID

STUDY NUMBER _____ PRINCIPAL INVESTIGATOR: Robert Walker, M.D.

STUDY TITLE: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Cytotoxic Syngeneic Lymphocytes in HIV Infected Identical Twins DONOR

9/21/93

INTRODUCTION

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you

OBJECTIVE OF THE STUDY

You are invited to participate in a study to evaluate the safety and side effects of administering genetically modified white blood cells (lymphocytes) to individuals with HIV-1 infection. You are invited to participate in this study because you have an identical twin who is infected with HIV-1 and because you test negative for HIV.

This study has been designed to evaluate the safety and tolerance of administering lymphocytes that have been removed from your blood, marked (or labeled) with a new gene in the lab, stimulated to grow to large numbers of cells in cell cultures, and then infused into your twin. We hope to learn not only about the safety of such cell transfers, but whether the gene we are introducing will help your twin clear HIV from his/her body. The potential benefit of participating in this study is that you may be contributing to the improved treatment of your identical twin by his/her receiving genetically modified, activated lymphocytes. The potential benefit to others would be the development of further knowledge about the use and side effects of this approach for treating HIV-infected patients, and possibly the development of a new strategy for treating HIV infection.

DESCRIPTION OF THE STUDY

After you and your twin have had screening blood tests, a tetanus booster injection, and a complete history and physical examination performed at the NIH, you will be scheduled to have a fraction of

your lymphocytes removed from the blood by a procedure called lymphapheresis. This will be performed in the Apheresis Unit of the NIH Blood Bank. During lymphapheresis, white blood cells are separated from the other constituents of blood. Blood flows out of your body via a catheter (a small plastic tube), which is usually inserted into an arm vein, and is separated into blood fractions using centrifugal force either manually or through a machine. The plasma (fluid component of blood) and red blood cells are returned to you via either the same or a second catheter, while the white blood cells are removed to be infused into your twin later. The procedure may take from 1 to 4 hours.

The lymphocytes removed by lymphapheresis will then be separated into CD8 (T8) cells and grown separately in the lab. After 1 to 4 days, a new gene will be introduced into the cells. The process involved in introducing the new gene is accomplished by first inserting the gene into a vector (i.e., an organism that carries material from one cell to another). The vector to be used in this study is prepared from a disabled mouse retrovirus -- that is, a virus that is not able to reproduce, and that normally infects only mice. The vector is mixed with the cells in the laboratory, enters the cells, and inserts the new gene into the cells' genetic material (chromosomes). Once inserted, the new gene will survive as long as the cell survives. The new gene that will be used will direct your lymphocytes to kill HIV infected cells in your twins body. In this way, the total viral burden may be reduced.

After the lymphocytes are marked with the new genes, they will be expanded in cell cultures up to approximately 1,000 times the original number of cells obtained. The time required to achieve these numbers of cells is approximately 3 weeks from the time you undergo lymphapheresis. Once the desired numbers of cells have been attained, some of the cells will be infused into your twin and some will be frozen for infusion at a later time. Your twin will come to the NIH Clinical Center to receive these cells by intravenous infusion. We plan to repeat the infusion process approximately every 8 weeks for up to 7 cell infusions. We plan to perform a single lymphapheresis at the beginning of the study and to freeze these cells for later expansion and gene modification. However, if complications arise (for example, contamination of the cells) and we decide not to work with these frozen cells, we may ask you to agree to additional lymphaphereses. We would not expect this to result in more than 2 or at most 3 separate lymphapheresis sessions for this study.

POTENTIAL RISKS

Complications of lymphapheresis include pain and bleeding at the catheter puncture site, fainting, temporary numbness and tingling, and machine malfunction. The most common form of machine malfunction is a leak or crack in the tubing through which the blood circulates. The risk of such an occurrence in our blood bank is 1 in 1,000 lymphapheresis sessions. Should this happen, you may lose as much as 1 unit (250 ml) of whole blood. One unit of blood is the standard amount taken during a blood donation.

In addition to the possible risks already mentioned, you will be subjected to the risks of blood drawing on your initial screening visit, and again within 2 weeks before the lymphapheresis. These include pain, redness, and swelling at the site of blood drawing, and rarely, fainting. Each time, about 2 to 10 tablespoons (30 to 150 ml) of blood will be drawn. The amount of blood drawn will not exceed 450 ml in a six-week period, which is within the guidelines set by the Clinical Center of the National Institutes of Health.

REQUIREMENTS FOR STUDY ENTRY

To participate in this study, your history, physical examination, and laboratory studies must show that you are able to provide informed consent and that you are in good health. If you have used drugs or alcohol to excess in the past, or if you are currently using these substances, you must agree to refrain from any further use and may be asked to participate in regular substance abuse counseling and/or therapy. If you are a woman capable of childbearing, a pregnancy test must be negative within 2 weeks prior to the lymphapheresis.

NIH AND CLINICAL CENTER POLICIES

There is no cost to you for the procedures associated with this study, but if you require medical care at other institutions or from other physicians during the course of this study, you or your insurance company will be charged for this care. Letters and results of tests performed at the NIH will be sent to your personal physician and you are encouraged to remain in close contact with your doctor. You must receive routine medical care from your own physician and you may not rely on the NIH for general medical care.

PREMATURE TERMINATION OF THE STUDY

In certain circumstances, some participants may need to terminate their participation in the study. You are free to withdraw at any time and for any reason without prejudice from the investigators. Serious side effects may cause you to be removed from the study at the discretion of the principal investigator. Other reasons for terminating your participation in the protocol include: noncompliance with clinic visits and other terms of the protocol; termination of the study by the principal investigator or the FDA; judgment of the principal investigator that it is in your best medical interest to leave the study.

HIV TESTING FREQUENCY AND POLICIES

As part of your participation in this study, it will be necessary to test your blood for the presence of infection by Human Immunodeficiency Virus. Testing of your blood for evidence of HIV infection will be performed prior to the time of lymphapheresis. Although you have been selected for this study because of a history of being HIV-negative, you should be aware of the following Clinical Center HIV Testing Policy in the event that your HIV test is found to be positive:

1. Your physician will notify you promptly of HIV test results.

2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.

3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them though an HIV counselor.

4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.

   a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.
   b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.
   c. The Clinical Center may report certain communicable diseases, including AIDS and symptomatic HIV disease, to the appropriate State and Federal government agencies.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor (496-8955).

STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

1. **Confidentiality.** When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of National Institutes of Health or Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

2. **Policy Regarding Research-Related Injuries.** The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the National Institutes of Health, Clinical Center, nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

3. **Payments.** If you are a patient, you are not paid for taking part in National Institutes of Health studies. Exceptions for volunteers will be guided by National Institutes of Health or Clinical Center policies.

4. **Problems or Questions.** Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Robert Walker, MD, or these other staff members also involved in this study: Richard T. Davey, Jr., MD; Michael Polis, MD; Judith Falloon, MD
Building 10, Room 11C103 Telephone: (301) 496-7196
National Institutes of Health
Bethesda, Maryland 20892

5. **Consent Document.** It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. **Adult Patient's Consent.**
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

_____
Signature of Adult Patient & Date Signed

B. **Parent's Permission for Minor Patient.**
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

_____
Signature of Parent(s) & Date Signed

(if other than parent, specify relationship)

_____
Signature of Investigator & Date Signed

_____
Signature of Witness & Date Signed

MEDICAL RECORD

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY
° Adult Patient or °Parent, for Minor Patient

INSTITUTE: NIAID

STUDY NUMBER _____ PRINCIPAL INVESTIGATOR: Robert Walker, M.D.

STUDY TITLE: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Cytotoxic Syngeneic Lymphocytes in HIV Infected Identical Twins RECIPIENT-A

9/21/93

INTRODUCTION

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DESCRIPTION OF THE STUDY

After you and your twin have had screening blood tests, a tetanus booster injection, and a complete history and physical examination performed at the NIH, your twin will be scheduled to have a

fraction of his/her lymphocytes removed from the blood by a procedure called lymphapheresis. This will be performed in the Apheresis Unit of the NIH Blood Bank. During lymphapheresis, white blood cells are separated from the other constituents of blood. The lymphocytes removed by lymphapheresis will then be separated into CD8 (T8) cells and grown separately in the lab. After 1 to 4 days, a new gene may be introduced into the cells depending upon a random assignment of treatments. The process involved in introducing the new gene is described in a separate consent form that you may also be asked to sign.

After the lymphocytes are marked with the new genes, they will be expanded in cell cultures up to approximately 1,000 times the original number of cells obtained. The time required to achieve these numbers of cells is approximately 3 weeks from the time your twin undergoes lymphapheresis. Once the desired numbers of cells have been attained, you will come to the NIH Clinical Center to receive these cells by intravenous infusion.

At the start of the study, you will be assigned in a random fashion to receive either genetically unmodified lymphocytes or genetically modified lymphocytes at one of three dose levels. Your chances of being assigned to one of the modified lymphocyte treatment groups is 75%. The study is divided into two treatment periods. In Period I you will receive a single infusion of cells followed by 8 weeks of observation. In Period II you will receive up to 6 additional infusions of cells of the same type as in Period I every 8 weeks. The cell dose in Period II may be adjusted up or down depending upon the results of Period I; therefore, there may be a delay of several weeks between Treatment Periods to allow for other patients to complete their treatment and observation.

For the first cell infusion, you will be admitted to the inpatient ward of the NIH Warren G. Magnuson Clinical Center. An intravenous catheter (a small plastic tube) will be inserted into an arm vein. However, if a suitable vein cannot be found, you may need to have a special intravenous catheter placed into a large vein in the neck or chest. This procedure would require a local anesthetic (lidocaine or Novocain) and would be performed by a physician in the intensive care unit. Once an intravenous catheter is established, the gene-modified cells obtained from your twin will be infused over a 60 minute period. During the infusion and for the next 24 hours, your vital signs (temperature, pulse, blood pressure, respirations), blood oxygen concentration, and urine output will be monitored regularly, as outlined in the protocol. Blood samples will be obtained from you at 1, 2, 4, and 6 hours after the start of the infusion to monitor for the presence of the gene modified cells. If there are no complications, you may be discharged from the hospital the following day, but you may need to return to the NIH as often as daily for the next 3 days for blood tests and then weekly as instructed.

For the next series of infusions, the identical schedule of tests and frequency of visits will be followed. However, if the first infusion was uncomplicated, subsequent infusions may be administered on an outpatient basis and monitoring of vital signs, etc., may last for 6 hours (rather

than 24 hours, as was done for the first infusion), at the discretion of the principal investigator. If any cell infusion is complicated by significant side effects, all subsequent infusions may be performed on an inpatient basis with monitoring for at least 24 hours post infusion, at the discretion of the principal investigator.

For the first week after each lymphocyte infusion, we will monitor on a daily basis (that is, Mondays through Thursdays only) CD4 counts, viral burden and p24 antigen levels, and the presence of the gene modified lymphocytes. Thereafter these tests and others monitoring blood and urine chemistry, blood counts, and markers of immune function will be obtained every two to four weeks. Six weeks after the final lymphocyte infusion has been administered, visits to NIH will be scaled back to monthly. Six months after the final lymphocyte infusion, your participation in the protocol will be completed and you will return to the sole care of your private physician.

POTENTIAL RISKS

The procedures involved in this study carry several potential risks. The risks can be divided into those related to the gene modification, those related to cell infusions, and those related to the other protocol procedures. The risks related to gene modification are discussed in a separate consent form that you will also be asked to sign.

The risks of infusing white blood cells, independent of gene modification, include fevers, chills, muscle aches, joint aches, local pain and redness at the site of infusion, skin rash, and severe allergic reactions. Depending on the severity of the reaction, the infusions may be slowed or stopped. In addition, medications such as aspirin, acetaminophen (Tylenol), ibuprofen (Motrin), meperidine (Demerol), or antihistamines may be administered for relief of symptoms. In the unlikely event that the cell cultures become contaminated with bacteria or fungus, infusion of the contaminated cells may lead to infection of the bloodstream that may spread to other organs. To further reduce the potential for this already unlikely event to occur, the cell cultures will be monitored regularly for contamination up to the time of infusion. In the event of a severe reaction to the cells, emergency life support and skilled personnel will be immediately available. Other theoretical, but extremely rare, risks include blood clots that may form in your veins, irregular heart beat, and sudden death.

It is possible that giving you activated cells may make your HIV infection worse rather than better, which could conceivably hasten the progression of your disease. In a small number of people with HIV infection who received activated CD8 cells on other protocols, no worsening was reported, but these results are still preliminary. In studies conducted at NIH, interleukin-2 infusions have been given to HIV patients for as long as 28 days without evidence of acceleration of their disease. Interleukin-2 is a lymphocyte activator, and giving it intravenously should produce a degree of lymphocyte stimulation similar to that which we hope to achieve on this protocol. Because of the

possible risk that infusing activated cells may make the HIV virus more active, we will be monitoring blood markers of viral activity and CD4 counts frequently. We will discuss these test results with you at regular intervals and will provide an ongoing assessment of the potential risks of your continued participation in the protocol. These ongoing assessments will be based on your own test results and on those of other participants in the study.

The placement of an intravenous catheter in an arm vein may be associated with localized pain and redness, and rarely, infection. If a central line must be placed into a larger vein of the neck or chest, additional risks must be considered. These include infection, local irritation about the incision sites, and rarely, bleeding in the chest cavity or a punctured lung. Should a central line be required, the potential risks will be discussed with you in greater detail and you will be asked to sign another consent form for that procedure.

If you have a history of an allergic reaction to cow's milk or other beef products, you will undergo a series of skin tests and an evaluation by the Allergy Consultation service at NIH. This is because the lymphocytes you are to receive, when grown in the lab, may be mixed with growth products derived from calf blood. The lymphocytes may therefore transfer a minute amount of calf products to you and, in an allergic person, this could result in a severe allergic reaction. During a skin test a small amount of liquid material is injected into the skin of the forearm. If your immune system shows a response to the test solution, a small bump similar to a mosquito bite may form at the site. Rarely, the site may become inflamed or ulcerated. There is also the rare possibility that you could develop a severe allergic reaction, known as anaphylaxis, to one of the test substances. This condition may be associated with low blood pressure, wheezing, difficulty breathing, and if left untreated, can even result in death. If anaphylaxis occurs, it usually does so within minutes of injection and responds to treatment with adrenaline and other medications.

In addition to the possible risks already mentioned, you will be subjected to the risks of multiple blood drawing. These include pain, redness, and swelling at the site of blood drawing, and rarely, fainting. Each time, about 2 to 10 tablespoons (30 to 150 ml) of blood will be drawn. The amount of blood drawn will not exceed 30 tablespoons (450 ml) in a six-week period, which is within the guidelines set by the Clinical Center of the National Institutes of Health.

We must emphasize that the technique of retroviral-mediated gene modification has not been used extensively in humans. Because this is such a newly introduced procedure, it is possible that despite all of our efforts, other unforeseen, perhaps serious and life-threatening, problems may occur.

OTHER AVAILABLE THERAPIES

Therapy for HIV infection is an area of intense research. Zidovudine (AZT) has been shown to be of benefit and is currently licensed for treating HIV infection in those with less than 500 CD4 cells. Dideoxyinosine (ddI) has been approved by the FDA for treatment of patients who either cannot tolerate or who are no longer responding to zidovudine. A third drug, dideoxycytidine (ddC), is also licensed for the treatment of HIV infection. in combination with zidovudine. In addition to these available antiviral drugs, preventing pneumocystis pneumonia (PCP) with oral trimethoprim-sulfamethoxazole or aerosolized pentamidine has also been shown to prolong the lives of patients at risk for PCP. Other potentially promising therapies for HIV infection are under study and may be available to you at this or another institution.

PREMATURE TERMINATION OF THE STUDY

In certain circumstances, some participants may need to terminate their participation in the study. You are free to withdraw at any time and for any reason without prejudice from the investigators. Serious side effects may cause you to be removed from the study at the discretion of the principal investigator. Other reasons for terminating your participation in the protocol include: the development of a life-threatening malignancy; noncompliance with clinic visits and other terms of the protocol; termination of the study by the principal investigator or the FDA; or judgment of the principal investigator that it is in your best medical interest to leave the study.

REQUIREMENTS FOR STUDY ENTRY

To participate in this study, your history, physical examination, and laboratory studies must show that you are able to provide informed consent and that you do not suffer from a serious emotional or psychological disorder, that you do not have lymphoma (a cancer of the lymph gland), and that your expected survival is greater than at least 6 months. If you have Kaposi's sarcoma (a skin cancer associated with HIV infection), you must have had this diagnosis confirmed by a biopsy specimen and must not have received radiation therapy, chemotherapy, or other intravenous or oral drug therapy for this tumor within 4 weeks of beginning lymphocyte infusions.

The effects of infusions of activated gene-modified lymphocytes on the developing fetus are unknown and might possibly be harmful. Therefore, if you are a woman capable of childbearing, you must have a negative pregnancy test within 2 weeks prior to entry into this study and again within 24 hours before receiving lymphocyte infusions. You also must agree to avoid pregnancy during your participation in this study through the use of effective birth control or abstinence. If you are a male of reproductive ability, you must agree to use effective barrier contraception or abstinence.

If you have used drugs or alcohol to excess in the past, or if you are currently using these substances, you must agree to refrain from any further use and may be asked to participate in regular substance abuse counseling and/or therapy.

You may be treated with licensed drugs or drugs available on "expanded access" for HIV-related conditions throughout your participation in this study. You may not take other experimental drugs while participating in this study.

Before participating in this study, you will be asked to appoint a person of your choosing as your "Durable Power of Attorney" or DPA. A DPA is a surrogate decision-maker, that is, someone who will make medical decisions in your behalf if and only if you become mentally impaired during the course of the study. A completed DPA form naming your surrogate decision-maker and signed by you will then become part of your medical record at the NIH.

NIH AND CLINICAL CENTER POLICIES

There is no cost to you for the procedures associated with this study, but if you require medical care at other institutions or from other physicians during the course of this study, you or your insurance company will be charged for this care. Antiviral therapies such as AZT, ddI, ddC, and PCP prophylaxis will not be provided by NIH and when indicated, must be prescribed by your personal physician. Once your participation in this protocol is completed, you will not be eligible for continuing care at NIH, but your doctors at NIH will be glad to provide telephone consultation to your home doctor at any time. Letters and results of tests performed at the NIH will be sent to your personal physician and you are encouraged to remain in close contact with your doctor. You must receive routine medical care from your own physician and you may not rely on the NIH for general medical care.

NIH AND CLINICAL CENTER HIV POLICY

Because you are known to be infected with HIV and will have tests performed to confirm this infection for the study, you should be aware of the following Clinical Center policy regarding the diagnosis of AIDS or HIV infection:

1. Your physician will notify you promptly of HIV test results.

2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.

3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them though an HIV counselor.

4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.

   a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.
   b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.
   c. The Clinical Center may report certain communicable diseases, including AIDS and symptomatic HIV disease, to the appropriate State and Federal government agencies.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor (496-8955).

STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

1. **Confidentiality.** When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of National Institutes of Health or Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

2. **Policy Regarding Research-Related Injuries.** The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the National Institutes of Health, Clinical Center, nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

3. **Payments.** If you are a patient, you are not paid for taking part in National Institutes of Health studies. Exceptions for volunteers will be guided by National Institutes of Health or Clinical Center policies.

4. **Problems or Questions.** Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Robert Walker, MD, or these other staff members also involved in this study: Richard T. Davey, Jr., MD; Michael Polis, MD; Judith Falloon, MD
Building 10, Room 11C103 Telephone: (301) 496-7196
National Institutes of Health
Bethesda, Maryland 20892

5. **Consent Document.** It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. Adult Patient's Consent.
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

_____
Signature of Adult Patient & Date Signed

B. Parent's Permission for Minor Patient.
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

_____
Signature of Parent(s) & Date Signed

(if other than parent, specify relationship)

_____
Signature of Investigator & Date Signed

_____
Signature of Witness & Date Signed

MEDICAL RECORD

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY
° Adult Patient or °Parent, for Minor Patient

INSTITUTE: NIAID

STUDY NUMBER _____ PRINCIPAL INVESTIGATOR: Robert Walker, M.D.

STUDY TITLE: A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Cytotoxic Syngeneic Lymphocytes in HIV Infected Identical Twins RECIPIENT-B

9/21/93

INTRODUCTION

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you

OBJECTIVE OF THE STUDY

This study has been designed to evaluate the safety and tolerance of administering lymphocytes that have been removed from your twin's blood, marked (or labeled) with a new gene in the lab, stimulated to grow to large numbers of cells in cell cultures, and then infused into you. We hope to learn not only about the safety of such cell transfers, but whether the gene we are introducing will help you clear HIV from your body. The potential benefit of participating in this study is that you may reduce your HIV viral burden after receiving genetically modified, activated lymphocytes. The potential benefit to others would be the development of further knowledge about the use and side effects of this approach for treating HIV-infected patients, and possibly the development of a new strategy for treating HIV infection.

DESCRIPTION OF THE STUDY

As part of your participation in the above study, you may undergo a procedure known as gene transfer depending upon a random assignment of treatments. Because gene transfer is a special procedure that has its own unique risks and precautions, you are being asked to sign a separate consent form.

After your twin sibling has had a portion of his/her blood cells removed by lymphapheresis, these

lymphocytes will be separated into CD8 (T8) cells and grown separately in the lab. After 1 to 4 days of growth, a new gene called [CD4-zeta] may be inserted into the cells.

The process involved in introducing the new gene is accomplished by first inserting the gene into a vector (i.e., an organism that carries material from one cell to another). In this study, the vector to be used is prepared from a disabled mouse retrovirus--that is, a virus that is not able to reproduce and that normally infects only mice. The vector is mixed with the cells in the laboratory, enters the cells, and inserts the new gene into the cells' genetic material (chromosomes). Once inserted, the new gene will survive as long as the cell survives. The new gene will be used to direct your twin's lymphocytes to kill HIV-infected cells in your body. In this way, the total viral burden may be reduced.

POTENTIAL RISKS

The risks related to gene transfer are theoretical, since the experience with gene marking and gene therapy experiments in humans thus far demonstrates no serious side effects, toxicities, or other ill effects due to gene modification of cells using retroviral vectors.

One potential risk relates to the vector. Even though the vector used to transfer the CD4-zeta genes into the cells cannot grow and is considered harmless to humans, it is possible that events could occur within the cell that would permit the vector to grow and/or make the cell cancerous. In human experiments, where more that 30 patients have received gene modified cells, no evidence for the presence of "growing vector" has been seen, and no lymphomas or other cancers related to the gene modified cells have occurred.

Another theoretical risk is that the new gene may, by randomly inserting into the chromosome, disrupt a gene essential for maintaining cell function and that particular cell might die.

We must emphasize that the technique of retroviral-mediated gene transfer has not been used extensively in humans. Because this is such a newly introduced procedure, it is possible that despite all of our efforts, other unforeseen, perhaps serious and life-threatening, problems may occur.

Since this study involves a new treatment approach to patients with HIV infection, it will be important to maintain contact with you so that we can monitor your overall progress and monitor for any possible long term side effects. In the event of your death, we would request an autopsy for the purpose of determining the cause of death.

MEDICAL RECORD | CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY | Continuation:
° Adult Patient or ° Parent, for Minor Patient | page 3 of 3 pages

STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

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3. Payments. If you are a patient, you are not paid for taking part in National Institutes of Health studies. Exceptions for volunteers will be guided by National Institutes of Health or Clinical Center policies.

4. Problems or Questions. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Robert Walker, MD, or these other staff members also involved in this study: Richard T. Davey, Jr., MD; Michael Polis, MD; Judith Falloon, MD
Building 10, Room 11C103 Telephone: (301) 496-7196
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COMPLETE APPROPRIATE ITEM BELOW, A or B:

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I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

_____
Signature of Parent(s) & Date Signed

(if other than parent, specify relationship)

_____
Signature of Investigator & Date Signed

_____
Signature of Witness & Date Signed

Robert E. Walker

Building 10, Room 11C103
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
(301) 402-0564
Fax (301) 402-4097

Academic appointments

1990-present	Senior Investigator, Intramural AIDS Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
1990-present	Senior Attending, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland
1991-present	Clinical Assistant Professor, Department of Medicine, Georgetown University Medical Center, Washington, D.C.

Postdoctoral training

1987-1990	Fellow in Pulmonary and Critical Care Medicine, Hospital of the University of Pennsylvania, Philadelphia
1986-1987	Staff Fellow, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
1984-1986	Resident in Internal Medicine/Primary Care, George Washington University Medical Center, Washington, D.C.
1983-1984	Intern in Internal Medicine, George Washington University Medical Center, Washington, D.C.

Education

1979-1983	M.D., University of Maryland School of Medicine, Baltimore
1975-1979	A.B., Vassar College, Poughkeepsie, New York; Major: English

Specialty certifications

1991 Diplomate, American Board of Internal Medicine, Critical Care Medicine
1990 Diplomate, American Board of Internal Medicine, Pulmonary Disease
1986 Diplomate, American Board of Internal Medicine, Internal Medicine

Professional affiliations

Member, American Thoracic Society
Member, District of Columbia Thoracic Society

Licensure

District of Columbia 14924
Maryland D41015
Virginia 0101038225
Pennsylvania MD-0393180E

Publications

Allegra CJ, Chabner BA, Tuazon CU, Ogata-Arakaki D, Baird B, Drake JC, Simmons JT, Lack EE, Shelhamer JH, Balis F, Walker RE, Kovacs JA, Lane HC, Masur H. Trimetrexate for the treatment of Pneumocystis carinii pneumonia in patients with the acquired immunodeficiency syndrome. New England Journal of Medicine 1987; 317:978-985

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HUMAN GENE TRANSFER PROTOCOL

9403-070 Brigham, Kenneth; Vanderbilt University, Nashville, Tennessee; *Expression of an Exogenously Administered Human Alpha-1-Antitrypsin Gene in the Respiratory Tract of Humans.*

Date of RAC Approval: 3-3-94
Date of NIH Approval: 10-25-94

Scientific Abstract

SCIENTIFIC ABSTRACT

We propose initial studies in humans with the long term goals of using gene transfer techniques for the treatment of patients with acute lung injury (adult respiratory distress syndrome--ARDS) and patients with alpha-1 antitrypsin (AAT) deficiency. We propose to use cationic liposomes to deliver a plasmid DNA construct with the cDNA for human alpha-1 antitrypsin driven by a cytomegalovirus promoter. Two different patient protocols are proposed using the exact same DNA and liposome preparations. In patients scheduled for elective pulmonary resection, we will instill plasmid/liposome complexes into a subsegment of the lung to be removed through a fiberoptic bronchoscope and obtain samples of the transfected portion and of the non-transfected portion of the lungs at the time of surgery. By immunohistochemistry, in situ hybridization, western and northern analysis, we will assess the amount and location of transgene expression in the lungs. Histological studies will also determine the effects of the intervention on lung structure. In patients with alpha-1 antitrypsin deficiency, we will instill plasmid/liposome complexes into one nostril and take serial nasal lavages from each nostril, measuring alpha-1 antitrypsin by ELISA in the lavage samples to assess expression of the AAT transgene. Cells obtained by nasal scraping will also be examined by immunohistochemical staining and in situ hybridization to assess transgene expression. Histological appearance of the cells will provide information about effects of the intervention on nasal epithelial cell structure. Because proteolytic events, principally a consequence of the inflammatory process, appear to be important in the pathogenesis of acute lung injury and of chronic pulmonary disease in patients with AAT deficiency (emphysema), development of safe and efficacious systems for delivering the AAT gene to respiratory tract cells might provide a new form of therapy for patients with these diseases.

Non-Technical Abstract

NON-TECHNICAL ABSTRACT

When inflammation occurs in the lungs, lung cells are injured in part by the release of enzymes from some white blood cells which digest proteins. Normally, a protein present in the lungs counteracts the damaging enzymes; this protein is called alpha-1 antitrypsin (AAT). Some people inherit a deficiency of AAT because the gene responsible for producing it is defective. These patients develop emphysema at an early age, presumably because the white blood cell enzymes digest the lungs. Also, when the lungs are injured as a result of blood stream infection, trauma or some other causes, this, too, appears to be in part because the white blood cells release the same enzymes which digest lung cells. In this case, the person may have normal amounts of AAT, but so much of the enzymes are released that this normal defense is overwhelmed. In either case (either AAT deficiency or acute lung injury), if lung cells could be genetically engineered to make increased amounts of AAT, the lung might be protected from injury. We propose to give the gene for AAT imbedded in some DNA which will cause the gene to make the AAT protein in large amounts to the lungs of humans. We will use DNA in a form which does not incorporate into host DNA (a circular form of DNA called a plasmid) and we will combine the plasmid with small fat globules called liposomes. These special liposomes are constructed so that they associate by charge interaction with DNA and escort the DNA into cells so that it can be expressed. In order to expose patients to minimal risk, we propose to begin by putting the plasmid/liposome into a lung that is going to be removed for some other reason (usually cancer) 2-3 days before surgery and then obtaining some of the removed lung at surgery in order to see whether there was any damage and also whether the gene we gave is working in the lungs. In some patients with AAT deficiency, we propose to give the plasmid/liposome to one nostril and then wash the nostrils each day to measure AAT in the fluid as a measure of whether the gene we gave is working. Some cells will also be taken from the nose by scraping and tested for the administered gene function. We have shown in cells in culture and in animals that the plasmid/liposome technology works and is safe; these studies will determine whether that is also true in the human respiratory tract. This information is critical to proceeding to any tests of therapeutic effectiveness of this approach.

1704

Brigham Protocol

HUMAN GENE TRANSFER PROTOCOL

EXPRESSION OF AN EXOGENOUSLY ADMINISTERED HUMAN ALPHA-1 ANTITRYPSIN GENE IN THE RESPIRATORY TRACT OF HUMANS

Principal Investigator: Kenneth L. Brigham, M.D.

Investigators: Angelo Canonico, M.D., James Stewart, M.D., Hans Schreier, Ph.D., Jon Conary, Ph.D., Leaf Huang, Ph.D.

Center for Lung Research, Vanderbilt University

Nashville, Tennessee 37232

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Curricula Vitae

Protocol

PROTOCOL FOR EXPRESSION OF AN EXOGENOUSLY ADMINISTERED HUMAN ALPHA-1 ANTITRYPSIN GENE IN THE RESPIRATORY TRACT OF HUMANS

Principal Investigator: Kenneth L. Brigham, M.D.

Investigators: Angelo Canonico, M.D., James Stewart, M.D., Hans Schreier, Ph.D., Jon Conary, Ph.D., Leaf Huang, Ph.D.

Center for Lung Research, Vanderbilt University

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Specific Aims

We wish to determine whether a human alpha-1 antitrypsin transgene in an expression plasmid vector can be introduced into the cells of the respiratory tract of humans using a cationic liposome delivery system and to determine the magnitude and time course of expression of the transgene. In addition, we will determine the structural effects of delivery of plasmid/cationic liposome complexes on the lungs and on nasal epithelium. Specifically, we propose:

1. To determine whether delivery of a plasmid containing the cDNA for human alpha-1 antitrypsin complexed to cationic liposomes to the nasal mucosa of patients with alpha-1 antitrypsin deficiency will result in expression of the alpha-1 antitrypsin transgene in nasal epithelial cells and in secretion of the alpha-1 antitrypsin protein into nasal fluid.

2. To define the time course of expression of a human alpha-1 antitrypsin transgene delivered to the nasal mucosa of patients with alpha-1 antitrypsin deficiency using cationic liposomes.

3. To determine effects of a single administration of plasmid/cationic liposome complex on the histological appearance of human nasal epithelial cells.

4. To determine whether delivery of a plasmid containing the cDNA for human alpha-1 antitrypsin complexed to cationic liposomes to the lower respiratory tract of patients scheduled for elective pneumonectomy will result in expression of the alpha-1 antitrypsin transgene in airway epithelium and/or other lung cells and synthesis and secretion of the alpha-1 antitrypsin protein by the lungs.

5. To determine effects of a single administration of plasmid/cationic liposome complex to the lower respiratory tract on the histological appearance of the lungs.

Background and Significance

Gene therapy in the lungs Most research in gene therapy in the lungs has focused on the two heritable disorders, alpha-1 antitrypsin deficiency and cystic fibrosis. Since the discovery of the cystic fibrosis transmembrane conductance regulator gene (CFTR), the principal driving force toward clinical application of gene therapy to the lungs has been cystic fibrosis.

The most extensively studied technology for in vivo delivery of transgenes to the lungs involves the use of replication deficient adenovirus vectors. Such vectors can deliver reporter genes as well as genes encoding either AAT or CFTR (1,2) to the lungs of animals. Based on that work, there are now several phase I clinical trials attempting to deliver the CFTR to patients with cystic fibrosis either intranasally or via bronchoscopy to the lower respiratory tract. Early reports from studies in humans indicate that nasal instillation is safe (3).

There are several potential problems with the adenovirus vector. Some investigators have seen an acute inflammatory response to the vector when delivered into the airways in animals (4). At least one patient in the clinical studies with intra-airway administration appears to have had a clinically important inflammatory response (5). Although replication deficient, the vector does involve delivery of viral proteins resulting in an immune response which could limit long term usefulness.

Adeno-associated virus (AAV) vectors might have some advantages over adenovirus. AAV constructs can be used to deliver functioning transgenes to the lungs in vivo. Flotte reported expression of the CFTR gene in airway epithelial cells of rabbits for several weeks following airway delivery of the gene in an AAV vector with no evidence of inflammation (6). Problems with this approach include difficulties in producing quantities of the AAV constructs which would be required clinically and at least theoretical problems resulting from incorporation of the vector into the host genome.

Liposome mediated DNA delivery in vivo In 1989, we initially demonstrated expression of a reporter gene in the lungs of animals following either intravenous or intra-airway delivery of plasmid/cationic liposome complexes (7). Since then several investigators have used this approach in experimental animals and, more recently, in phase I studies in humans.

In both rabbits and cotton rats, delivery of reporter genes and of human CFTR to the lungs has been demonstrated following airway instillation or aerosol delivery of cationic plasmid/liposome complexes (8-11). Very high transfection efficiencies have been reported in airway epithelial cells (from 40% to 70% (8)) in the absence of demonstrable toxicity. In the transgenic mouse model of cystic fibrosis, delivery of a CFTR containing plasmid in this manner produced expression of the CFTR in the lungs and partial correction of the chloride transport defect (10). Our earlier observation that intravenous delivery of plasmid/liposome complexes was not toxic and resulted in transgene expression in the lungs has been confirmed by others (11).

Delivery of plasmid/cationic liposome complexes to the lungs, at least over short periods, does not alter lung structure or function. Following aerosol delivery of a plasmid using cationic liposomes, Debs saw prolonged expression of a reporter gene in the lungs of mice without toxicity (8). Other kinds of liposomes have been delivered to humans by aerosol with no alteration in lung function (12). We have delivered plasmid/liposome complexes to rabbits repeatedly for four weeks by both aerosol and intravenous injection and have seen no effects on lung mechanics, arterial blood gases or lung structure (13).

Some phase I studies using cationic liposomes to deliver DNA to humans are now underway in this country and in England. Nabel and coworkers have used this technology with direct injection into melanomas in humans (14,15). More recently, the same group has injected the plasmid/cationic liposome complexes into the pulmonary artery of patient with lung cancer and observed no adverse effects (16). Initial studies using either intranasal instillation or aerosol administration of plasmid containing the CFTR gene complexed to cationic liposomes are now underway in England.

Several different cationic liposome preparations are available which vary some in their ability to deliver DNA and, potentially, in their in vivo effects. The preparation which is being used in the human studies currently approved in this country are DC-chol liposomes, originally synthesized by Dr. Leaf Huang. Dr. Huang is a co-investigator in the studies we propose here and he will produce and supply DC-chol cationic liposomes identical to those being used in human studies by Nabel.

DNA constructs for in vivo use Investigations of in vivo gene transfer to the lungs have used either viral vectors or liposome associated plasmids. As discussed above, replication deficient adenovirus vectors have received much attention and are currently being used in preliminary studies in humans. Adeno-associated virus vectors have also been shown capable of delivering DNA to respiratory epithelium and may have less toxicity than adenovirus vectors (17).

Although cationic liposomes can deliver any nucleic acid construct, most work with this technology for in vivo delivery has concentrated on delivery of DNA in the form of plasmids. Plasmids associate readily with cationic liposomes by charge-charge interaction and, at least in lung cells in culture and in vivo, cationic liposomes deliver functioning plasmids effectively. Plasmids do not cause human disease, are easy to work with in the laboratory and can be produced in large quantities. Although plasmids do not readily incorporate into host DNA and do not replicate in mammalian cells, some investigators have found expression of transgenes imbedded in plasmids for several weeks following transfection (8).

Rationale for alpha-1 antitrypsin gene therapy An inherited deficiency of the principal endogenous antiprotease, alpha-1 antitrypsin (AAT), results in development of emphysema and respiratory failure at an early age (18). Although the exact incidence of abnormalities in the AAT gene is not known, it now appears certain that the disease is not rare. Current therapy consists of parenteral administration of the AAT protein at monthly intervals, although whether this treatment alters the course of the lung disease in these patients is unclear. Disadvantages of current therapy include expense, the necessity of repeated intravenous infusions and reactions to the protein either because of impure preparations or due to immunologic responses.

We believe that if a functioning human AAT gene could be introduced into lung cells accessible through the airways, that the resultant local production of the antiprotease might be sufficient to protect the lungs from proteolytic injury, even though the total amount of AAT produced might be less than usually made in the liver. If the treatment is safe and efficacious, aerosol administration of plasmid/liposome complex at intervals probably in excess of a week would be trivial therapy compared to that currently available.

In addition to the treatment of patients with AAT deficiency, we are interested in expanding the application of gene therapy to the treatment of acute lung injury. Acute lung injury resulting in the clinical entity called the adult respiratory distress syndrome (ARDS) occurs in approximately 150,000 Americans each year and mortality remains in excess of 50%. Knowledge accumulated over the past several years implicates the inflammatory response, particularly neutrophil activation, as important in the pathogenesis of lung injury. Neutrophils injure lung cells in part because of release of neutrophil elastase which is inactivated by AAT (19,20,21). In animal models and cultured cells, there is evidence that AAT can prevent injury of lung cells consequent to inflammation (21,22). Thus, transient hyperexpression of the AAT gene in the lungs of patients with or at risk for developing acute lung injury could be preventive or therapeutic.

Because of our ultimate therapeutic goals, we propose to determine whether expression of an AAT transgene can be achieved using a plasmid/liposome delivery system in both patients with AAT deficiency and in patients without AAT deficiency. In the former case, we choose to begin with nasal instillation because there is already clinical experience with this approach, cells from the nasal mucosa can be easily obtained, no invasive procedures are necessary and AAT concentrations in nasal wash fluid can serve as a convenient and simple monitor of transgene expression. In the latter case, we propose to select patients who are to undergo elective lung resection, most commonly for cancer, and to instill the plasmid/liposome into the lung which is to be removed surgically. We believe that this approach has the dual advantage of minimizing any risk to the patient and of obtaining lung tissue which will permit more detailed assessment of transgene expression and toxic effects of plasmid/liposome delivery to the lower respiratory tract. We believe that this experimental design is conservative in that it minimizes risk and maximizes the information which can be obtained from the human studies.

Preliminary Studies

Appended are 4 reprints and 2 manuscripts currently under review which present a large amount of the information from our laboratories which is relevant to this proposal. Some of those data will be summarized briefly here and additional data will be discussed in more detail.

Liposome delivery of drugs to the lungs One of us (H. Schreier) has worked with liposomes as a drug delivery system for many years. That body of work demonstrates that liposomes can be delivered by aerosols of a size that reach the lower respiratory tract (P1) and that drugs can be effectively delivered to the lungs by this delivery technology (P2). Extensive studies of the safety of aerosol delivery of liposomes to the lungs of experimental animals and humans have shown no evidence of toxicity, even with repeated delivery of heavy liposome doses (P3,P2). Dr. Schreier's work also demonstrates the ability to target liposomes by altering their surface characteristics, including targeting to respiratory epithelial cells by incorporation of the attachment and fusion proteins from respiratory syncytial virus into the liposome surface (P1).

In vivo delivery of DNA using cationic liposomes Beginning in 1989, we have conducted a series of studies using cationic liposomes to deliver plasmids to the lungs. We have assessed safety and efficacy of this technology with the goal of clinical application.

In mice, we showed that either intravenous or intra-tracheal injection of plasmid/cationic liposome complexes resulted in expression of a chloramphenicol acetyltransferase (CAT) gene in the lungs (P4). Table I summarizes data from those studies. With the particular DNA construct which we used, we found that CAT expression was limited to the lungs, was related to the dose of DNA delivered and was higher in the lungs when the DNA was given intra-tracheally than when given intravenously. We saw no gross evidence of toxicity of the procedure in those studies.

TABLE I

CAT Activity in Lungs, Liver and Kidneys 72 hrs Following DNA-Liposome Injection into Mice

% chloramphenicol acetylation per h per μg protein x 10^{-2}

Route	Lungs	Liver	Kidneys
Intravenous			
30 μg DNA per mouse	24.5	0	0
15 μg DNA per mouse	10.1	0	0
Intratracheal			
30 μg DNA per mouse	35.7	0	0
Intraperitoneal			
30 μg DNA per mouse	0	0	0

A subsequent study in mice demonstrated expression of a gene encoding human growth hormone (hGH), a physiologically relevant secreted human protein, in the lungs of mice given plasmid/cationic liposome complexes intravenously (P8). As shown in the appended reprint, hGH gene expression required that the plasmids be complexed to cationic liposomes and was essentially limited to the lungs.

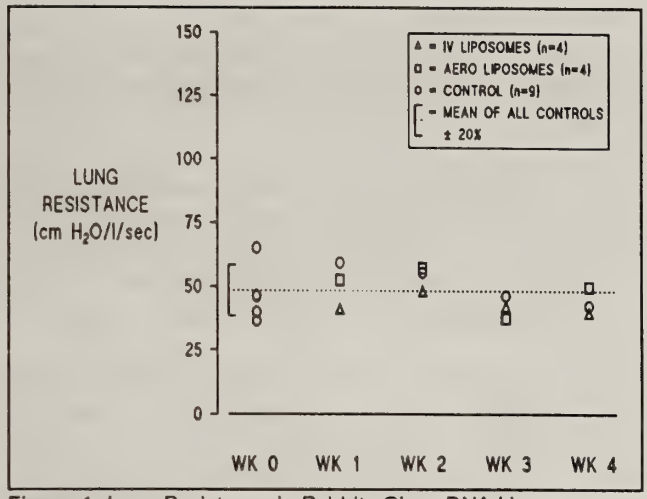

Figure 1: Lung Resistance in Rabbits Given DNA-Liposomes

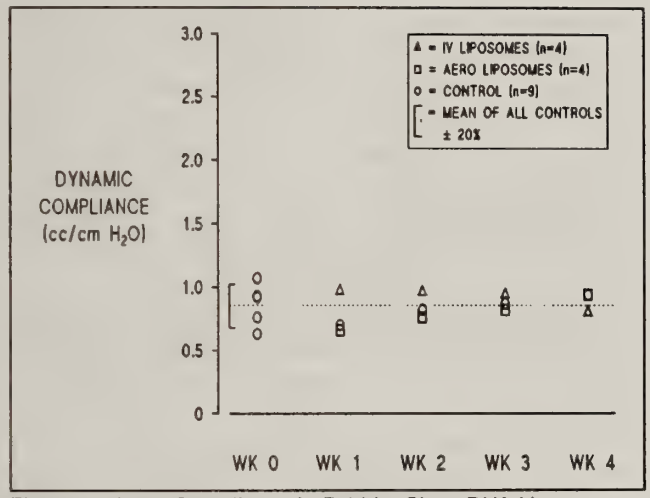

Figure 2: Lung Compliance in Rabbits Given DNA-Liposomes

We constructed a plasmid vector using a CMV promoter to drive a human alpha-1 antitrypsin gene and tested expression in the lungs of rabbits after either intravenous or aerosol delivery of the plasmid complexed to cationic liposomes (P9,P10). This is the same plasmid construct which we propose to use in the studies in humans. A manuscript describing the initial work is in press and a copy is appended. We found that after either intravenous or aerosol delivery of plasmid/cationic liposome complexes, the AAT transgene was expressed in the lungs. Presence of the protein was established by western blots and immunohistochemistry using an antibody which did not crossreact with rabbit proteins. Transgene message was detected by Northern analysis of lung RNA using a specific probe. Following delivery to the airway, transgene expression was predominantly in the lungs and immunohistochemical staining showed abundant AAT protein throughout the airway epithelium. Autoradiograms of lung tissue sections following administration of a radiolabelled plasmid suggested that about 10-20% of cells contained the plasmid. Expression could be demonstrated at least up to a week following DNA administration. None of the animals showed any evidence of toxic effects of the procedure.

These studies document that foreign genes can be delivered in the form of plasmids to the lungs using cationic liposomes as the delivery vehicle and that the procedure is not obviously toxic to the animals.

Effects of plasmid/cationic liposome administration on lung structure and function To assess the possible toxicity of plasmid/liposome to the lungs in more detail, we conducted an additional study in rabbits. A manuscript reporting these studies is under review and is appended (P11,P12). We gave rabbits plasmid/cationic liposome either intravenously or by aerosol at weekly intervals for 4 weeks, comparing their lung function and structure with that of a cohort of identical animals which received no intervention at all. At weekly intervals, one rabbit from each group was studied. We measured arterial blood gases and lung compliance and resistance, then killed some animals and observed histology of the lungs and other organs. Figures 1 and 2 show the time course of lung mechanics in each of the groups. Weekly administration of plasmid/liposome by either intravenous or aerosol route had no effect on

lung mechanics. Likewise, we saw no effects on arterial blood gases or histology of the lungs or other organs. There were no clinically apparent toxic effects of the intervention. These studies indicate that the intervention is not toxic in any way detectible by our experimental methods.

Protection of the lungs from endotoxin by gene therapy To determine whether transgene expression of sufficient magnitude to affect a pathophysiological response was possible using the plasmid/cationic liposome delivery system, we determined the effects of gene delivery to the lungs of rabbits on the response of the lungs to endotoxin perfusion. A manuscript reporting these studies is under review and a copy is appended (P13,P14).

Based on a large amount of work detailing the pathogenesis of the endotoxin response in the lungs over the past 20 years, we hypothesized that transfection of the pulmonary endothelium with a plasmid which would express the prostaglandin G/H (PGG/H) synthase gene would cause increased production of prostacyclin and PGE_2, both prostanoids which have anti-inflammatory actions, and that this would result in resistance of the lungs to the effects of endotoxin (P15). We inserted the cDNA encoding ovine PGG/H synthase into the same pCMV4 vector (now $pCMV_4$-PGH) which we used to study AAT, administered the plasmid complexed to cationic liposomes by intravenous injection to rabbits and 24 hours later, made an in situ homologous blood perfused lung preparation and tested the response of the lungs to addition of endotoxin to the perfusate. Control studies in random order were done on rabbits which were transfected with the empty vector using identical procedures to the experimental group. We found that lungs removed from rabbits transfected with the $pCMV_4$-PGH plasmid 24 hours following transfection produced increased amounts of both prostacyclin and PGE_2. Plasma urine concentrations of prostacyclin metabolite measured by gas chromatography/mass spectrometry in PGH transfected rabbits was double that in control animals. As shown in Figure 3, the pulmonary hypertensive response to endotoxin perfusion was almost totally abolished in the PGG/H transfected rabbits. Measurements of lung wet/dry rations at the conclusion of the experiments also showed prevention of endotoxin induced pulmonary edema in the PGG/H transfected group (W/D ratio= 4.8+/-0.3 in controls and 8.4+/-1.8 in the PGG/H transfected group $p<0.05$). To our knowledge, these are the first studies demonstrating a therapeutic effect of in vivo gene transfer in an animal model of lung injury. The experiments show that it is possible to obtain sufficient transgene expression using a plasmid/cationic liposome delivery system to alter physiologic responses.

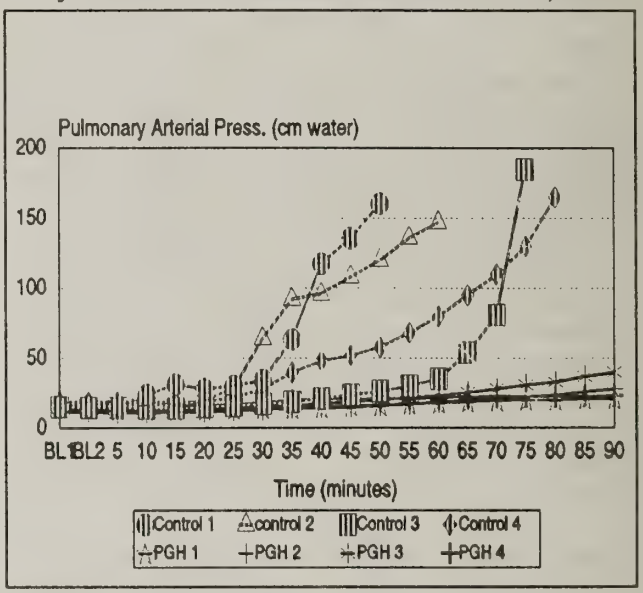

Figure 3: PGH Transfection Blocks PA Pressure Response to Endotoxin in Rabbits

In vivo delivery of plasmid/cationic liposomes to the lungs of sheep In order to obtain more information about the physiologic effects of administration of plasmid/liposome complexes and to assess efficacy in a larger animal, we have completed preliminary studies in sheep. Yearling sheep (30-40kg, body weight) were prepared surgically with chronic instrumentation which permits measurement of lung mechanics and pulmonary and systemic hemodynamics. This is a preparation with which we have worked with for the past 20 years and have described multiple times in the literature (P16). The preparation permits extensive

monitoring during an intervention done with a fully awake, unmedicated animal. After the sheep recovered from surgery, a baseline response to endotoxin infusion (0.5μg/kg) was measured. One week later, we infused intravenously 8mg DNA of the same $pCMV_4$-PGH plasmid which we used in the rabbit studies described above and in the appended manuscript (P13). The plasmid was complexed with 24mg cationic liposome (Lipofectin^R) prior to infusion. Pulmonary and systemic hemodynamics and lung mechanics were monitored throughout and following the infusion. During plasmid/liposome administration there was no evidence of distress in the animal. The only physiologic effect observed during infusion of the plasmid/liposome complex was a slight increase in pulmonary artery pressure which was directly related to the infusion rate. Slowing the infusion rate eliminated this response.

Twenty four hours following administration of the plasmid/liposome complex, endotoxin (0.5μg/kg) infusion was repeated and the two responses compared. Figure 4 shows the alveolar to arterial oxygen gradient over the course of the endotoxin response in the two studies conducted in this animal (i.e. before and after PGG/H gene transfer). Following transfection, the hypoxemia consequent to endotoxin infusion was substantially decreased. Similar results were seen for comparisons of lung mechanics. As shown in Figures 5 and 6, lung tissue removed from this animal at the end of the endotoxin experiment generated increased amounts of PGE_2 and decreased amounts of thromboxane. These results are like those which we saw in the rabbit experiments. During the DNA/liposome administration, there was no evidence of any distress in the animal.

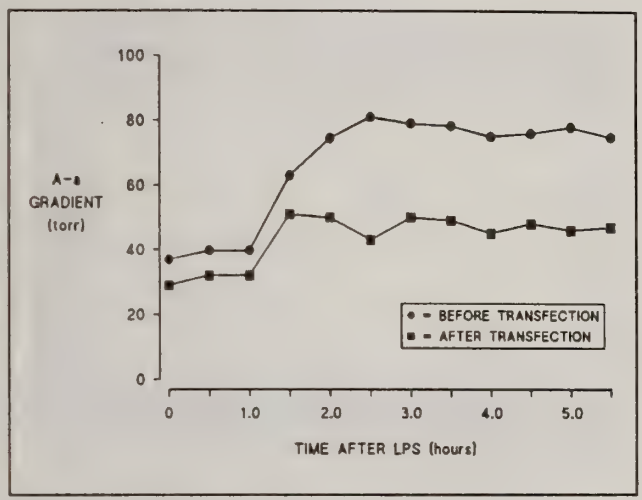

Figure 4: A-a DO_2 Responses to Endotoxin in a Sheep before and after PGH Synthase Gene Transfection

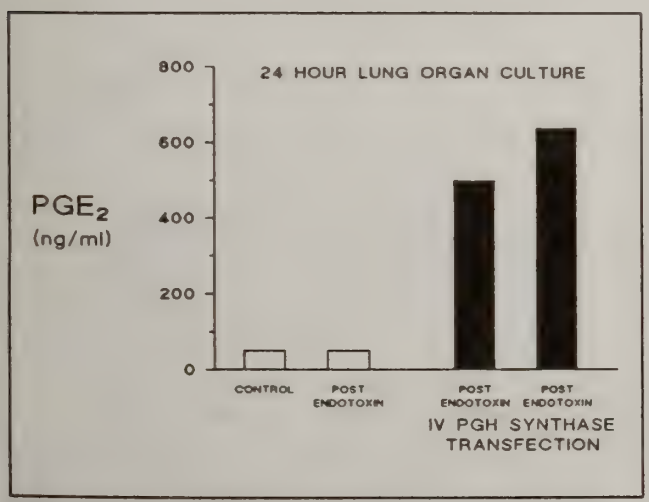

Figure 5: PGE_2 Production by Sheep Lung in Organ Culture

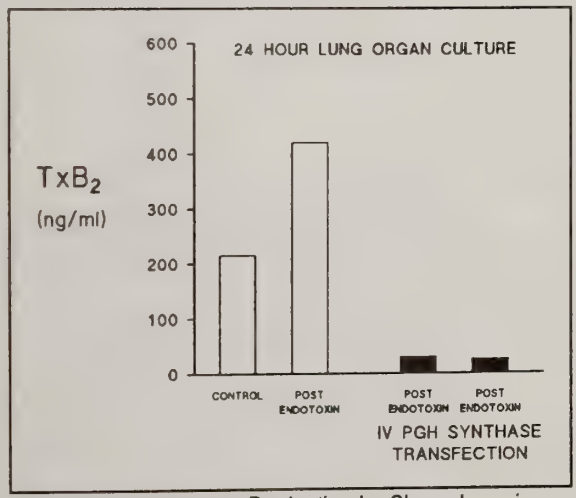

Figure 6: Thromboxane Production by Sheep Lung in Organ Culture

We have also performed essentially the exact study which we propose in humans scheduled to undergo elective lung resection in sheep. Through a fiberoptic bronchoscope wedged into a subsegment of the right lung, we instilled 500μg DNA of the pCMV$_4$-AAT plasmid complexed to 2500μg cationic liposomes (Lipofectin^R). There were no complications associated with the procedure and no evidence of distress of any kind. Twenty-four hours later, we killed the animal, removed the lungs and carried minces of the transfected lobe and a subjacent control lobe in organ culture for 24 hours. We performed Western blots of the supernatant culture fluid using an antibody specific for human alpha-1 antitrypsin and found clear evidence of human alpha-1 antitrypsin in the supernatant of the transfected lobe organ cultures. Only a faint band was detected from the subjacent lobe (Figure 7).

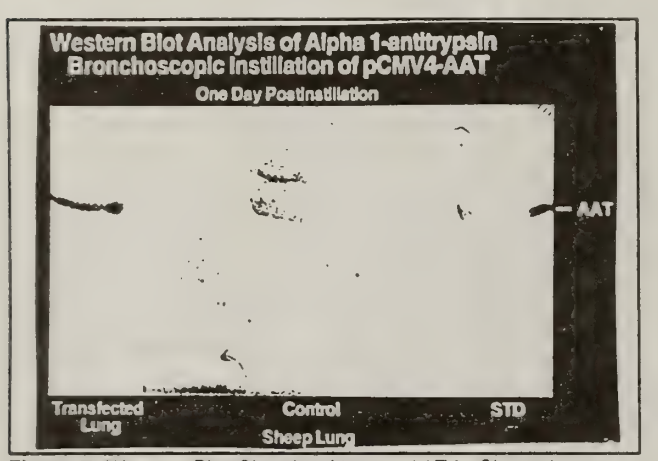

Figure 7: Western Blot Showing Human AAT in Sheep Lung Lipofected In Vivo

In summary, our studies in intact animals, including chronically instrumented unanesthetized sheep indicate that the procedures which we propose are harmless and result in substantial in vivo transgene expression.

Alpha-1 antitrypsin concentrations in nasal wash of normal humans Using the same protocol that we propose in this study, we have performed nasal washes in normal volunteers and measured alpha-1 antitrypsin concentrations by ELISA. Table II summarizes the data. Normal humans have substantial amounts of immunoreactive alpha-1 antitrypsin in their nasal lavage fluid which is easily measured. AAT measurement in nasal lavage fluid is a simple, painless and safe procedure which provides quantitative data related to alpha-1 antitrypsin status. Since there is already experience with nasal instillation of DNA in patients with cystic fibrosis and since alpha-1 antitrypsin is a secreted protein, serial monitoring of transgene expression by simply measuring AAT in nasal lavage fluid minimizes any possible risk to the patient while providing a large amount of information about the efficacy of the delivery technology which we propose to test.

Table II. Immunoreactive alpha 1-antitrypsin in serial nasal lavage (ng / 0.1 ml)

Day 1	Day 2	Day 3	Day 4
38788 ± 9118*	14776 ± 3685†	12505 ± 2234†	14267 ± 4191†

mean ± S.E.M.; *N=6, †N=7

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Research Design and Methods

Plasmid description and production of plasmid DNA

The $pCMV_4$ vector (23) was selected for the expression constructs because it represents a "state of the art" expression vector. In particular, this vector provides a stable mRNA through the use of human growth hormone termination and poly-adenylation signals. This vector also provides sequences from the alfalfa Mosaic Virus-4 which act as a translational enhancer by decreasing the requirement for initiation factors in protein synthesis. In addition, this construct also contains a polylinker region and the promoter-regulatory region of the human cytomegalovirus major immediate early gene.

The $pCMV_4$-AAT plasmid was constructed by linker-primer polymerase chain reaction (LP-PCR). The coding sequence of PGH synthase was inserted into the $pCMV_4$ expression vector. This method consists of synthesizing two oligonucleotide primers (20-30 nucleotides in length). One oligonucleotide is homologous to the 5' untranslated region immediately upstream (5') of the initiation codon and the second oligonucleotide is complementary to the 3' untranslated region immediately downstream (3') of the stop transcription codon. Both oligonucleotides have a one or two base substitution which creates a unique restriction enzyme site in the untranslated regions of the amplified gene. The 5' and 3' oligonucleotides were designed such that the created restriction enzyme site is approximately 8 nucleotides downstream from the 5' end of the oligonucleotide. Both of these requirements are critical, the former to insure a restriction enzyme site which is recognizable and cleavable and the latter to insure that the reading frame of the gene is not altered.

The reading frame of the gene of interest was amplified using Vent DNA polymerase, 100 ng of target DNA, a programmable temperature cycler, and standard reaction conditions (denaturing at 93.5^0, annealing at 56^0 and extension at 75^0). Vent DNA polymerase was used because it has a 3' to 5' proofreading activity in addition to enhanced stability at high temperature and a highly specific and processive 5' to 3' DNA polymerase activity. After PCR amplification, the unique restriction sites were cleaved with the appropriate restriction enzymes, the amplified gene was separated from the small fragments released by the action of the restriction enzymes and from unincorporated primers and nucleotides by gel filtration through a S-400 spin column. The amplified genes which now had cloning sites on each end were ligated into $pCMV_4$ which had been previously cleaved with the same restriction enzymes which were utilized to prepare the cloning sites on the amplified gene.

After ligation, the $pCMV_4$-AAT construct was transfected into fresh competent bacteria (*E. coli* NM522). The competent bacteria were prepared by standard methods (24). After transfection the bacteria were plated out on plates containing carbenicillin, an ampicillin analog which provides selection pressure for bacteria containing the $pCMV_4$ construct. The plasmid carries the gene for ampicillin resistance. After the bacteria which harbor the plasmid are have grown into distinct colonies, several of the colonies are grown up as individual 5 ml liquid cultures. An aliquot of the liquid cultures is stored and the rest is processed as a "mini" prep. We then confirm that the isolated plasmid contains the inserted piece of DNA by performing both a dot blot analysis and by releasing the inserted piece of DNA by performing a restriction enzyme digest. The restriction enzyme digest should yield both the linearized plasmid and the original piece of DNA.

Once a colony has been isolated which contains the piece of cloned DNA, a large scale plasmid preparation is grown. The plasmid is purified using the commercial Giga-prep column method (Quiagen). Finally, the isolated plasmid is precipitated with ethanol and resuspended in sterile water. Samples are stored at -20 °C in 1 ml aliquots.

For the studies proposed here, a large batch of plasmid (1.3-1.4 kg cells) will be prepared at the University of Iowa College of Medicine, Department of Microbiology, Iowa City, Iowa 52242 (Lacy Daniels) according to the following protocol. One 100 liter fermentor run is performed with *E. coli* strain NM522-pCMV$_4$-AAT grown in medium composed of a mixture of Luria Broth Base and Terrific Broth. An inoculum is prepared from an isolated colony, scaled up by growth on plates and grown finally in Fernbach flasks for the inoculum. All media contain originally ca. 75-100 μg ampicillin/ml. Cells are harvested with a Sharples continuous centrifuge after absorbance reaches a near maximal point. Cells are frozen directly in liquid nitrogen and shipped on dry ice. Cell yields are usually 1.3-1.4. kg wet cells per fermentor run.

Liposome production

DC-Chol/DOPE liposomes are prepared by one of the investigators, Dr. Leaf Huang, at the Department of Pharmacology, School of Medicine, at the University of Pittsburgh, 13th Floor, Biomedical Science Tower, Pittsburgh, PA 15261. DOPE is obtained from Avanti Polar Lipids, Inc., 700 Industrial Park Drive, Alabaster, AL 35007. DOPE has a Drug Master File Number with the U.S. FDA. The microfluidizer (model 110-S) was purchased from Microfluidics Corp., Newton, MA.

DC-Chol and DOPE (in chloroform) are mixed in a 6:4 molar ratio in a 250 ml round bottom flask. The lipid mixture is evaporated to dryness in a stream of nitrogen and allowed to vacuum desiccate (for at least 2 hours) to remove any residual chloroform. The thin lipid film is hydrated overnight (at least 12 hours at 4°C) with sterile water. Upon sufficient hydration, the flask is briefly sonicated to remove the lipid form from the walls of the flask. The hydrated lipids are shaken to disperse the lipids in the aqueous solution.

The microfluidizer is powered by compressed nitrogen gas and is operated at a pressure of 80 psi. The liposomes are cycled through the microfluidizer several times (5-15 passes) to reduce the mean diameter of the liposomes. The size of the liposomes is monitored by quasi-elastic light scattering (QLS). The liposome size is reduced by cavitation which occurs in the interaction chamber. This chamber is packed in ice to eliminate any heat which may be produced during operation of the microfluidizer.

Once the liposome size is reduced below 200 nm, the liposomes are passed through a 0.22 μm nylon filter to sterilize the liposomes.

The liposomes are assayed for phosphate content by a calorimetric method according to Bartlett (25) and diluted to a final concentration of 2 μmol of total lipids per ml.

The liposomes are packaged in 4 ml Wheaton screw cap vials which have been autoclaved. The packaging occurs in a sterile hood and the headspace of the vials is filled with nitrogen to prevent oxidation of the DOPE. The vials are stored a 4°C until use.

Liposomes will be sent by overnight express mail to one of the investigators at Vanderbilt, Dr. Hans Schreier, who will receive the shipment, document date, declaration (content, lot number) and amount of shipment received, and will store the material at 4°C in a refrigerator in the Liposome Laboratory of the CLR (Room B 1222 MCN).

Generation of plasmid/liposome complexes

DNA and liposomes will be mixed together at 37°C in a plasmid/liposome w:w ratio of 1:3 in sterile, pyrogen free polystyrene tubes and incubated for 30 minutes prior to administration.

Patient protocols

Patients undergoing lung resection We have designed the protocol to insure, as much as possible, safety to the patients being studied. The purpose of this study is to determine whether the human alpha 1-antitrypsin gene in a plasmid construct delivered with a liposome vehicle via the airways into the lungs of humans will function in such a way that the human

alpha 1-antitrypsin gene is expressed in the lungs. Several factors will maximize safety for the patients. First, we propose to use a plasmid construct. A plasmid is a circular piece of DNA which does not incorporate into the genome of mammalian cells and the plasmid which we use is not propagated in mammalian cells. Thus the risk of alteration of the host genome or creating any other abnormalities in DNA function should be minimal. Second, we propose to select patients who are scheduled for an elective pneumonectomy or lobectomy and to specifically instill the DNA/liposome complexes only in the lung to be removed at surgery. Again, this approach should minimize any potential risk to the patient of administering the DNA/liposome complexes by limiting it as much as possible to tissue which is to be removed within 72 hours.

We propose to select patients scheduled for an elective pneumonectomy or lobectomy whose chest radiographs show substantial amounts of normal lung tissue in the area to be removed. Seventy-two hours prior to when surgery will be done, a fiberoptic bronchoscopy will be performed in which the bronchoscope is wedged into a subsegment of the portion of the lung to be removed. Into that subsegment, DNA/liposome complexes will be injected. The DNA will be a plasmid vector containing the coding region for human alpha 1-antitrypsin driven by a cytomegalovirus promoter region. This plasmid has been developed in our laboratories at Vanderbilt and is the same plasmid which we have used extensively in the animal studies discussed above. The material will be prepared in our laboratory in a manner that insures the elimination of endotoxin. $pCMV_1$-AAT DNA, 800-2400 μg, will be complexed with the liposome, DC-Chol/DOPE (4000-12000 μg) and incubated at room temperature for 15 to 30 minutes prior to injection into the lung. To identify the location of the injected DNA, a small drop of methylene blue will be placed in the micosa at the end of the bronchoscope. Following injection of the DNA/liposome complexes, the bronchoscope will be removed. A 10 ml blood sample obtained from an antecubital vein will be obtained at the time of the study as well as 48 hours later. Liver function tests, a complete blood count, and alpha 1-antitrypsin values will be compared between the two blood sample. A chest radiograph will be obtained at 48 hours to assess any evidence of pneumonia.

At the time of surgery, lavage of the transfected area will be performed and compared to a lavage obtained from a control untransfected region of the lung. Lavage fluid will be analyzed for difference in alpha 1-antitrypsin levels as well as difference in the cell differential. Following removal of the lung, two to five gram samples of lung from the transfected subsegment as well as an untransfected section will be taken with the remainder of the lung remaining with the pathologist.

One portion of the two lung samples will be fixed in formalin and another fixed in glutaraldehyde for examination by light and electron microscopy and for in situ hybridization studies. Another portion of the lung will be minced and incubated in organ culture in medium 199. After 24 hours, the medium will be collected for measurement of human alpha 1-antitrypsin by an ELISA assay and by Western blot. Another portion of each lung sample will be processed for the isolation of both DNA and RNA. DNA analysis will be done by probing for sequences peculiar to the plasmid by Southern blotting. Northern blot analysis and/or RT-PCR will be done to detect human alpha 1-antitrypsin mRNA. Since alpha 1-antitrypsin is not normally synthesized in the lung tissue, presence of RNA transcripts for alpha 1-antitrypsin should confirm successful gene expression. Histological examination will include immunohistochemical staining of human alpha 1-antitrypsin for the detection of alpha 1-antitrypsin protein and in situ hybridization for location of mRNA transcripts as well as routine light and electron microscopic examination to assess structural effects of the intervention.

We propose to conduct these experiments initially in five humans.

Patients with alpha-1 antitrypsin deficiency Patients will be selected who have documented alpha 1-antitrypsin deficiency (serum concentration less than 80 mg/dl). Baseline nasal lavage will be performed to measure pretreatment alpha 1-antitrypsin level. The patient

will place his chin to his chest, occlude his nares with either his free hand or with nose clips and 3 ml saline will be infused into the nasal cavity using a 21 gauge butterfly with the needle removed. After infusion, occlusion of the nose will be terminated and the lavage fluid collected by gravity drainage into a 50 ml conical tube.

After establishing baseline alpha 1-antitrypsin level in the nasal lavage, DNA/liposome complex (400-600 μg DNA + 1600-3000 μg DC-Chol/DOPE) in sterile distilled water will be infused into the nasal cavity as outlined above. The nares will remain occluded for 30 minutes to maximize gene transfer. After 30 minutes, occlusion will be terminated and the DNA/liposome mixture collected by gravity drainage into a 50 ml conical tube. At day 3, 5, and 7, lavages will be performed to measure alpha 1-antitrypsin levels after gene transfer. In addition, nasal scrapings of epithelial cells will be performed for measurement of human alpha 1-antitrypsin DNA, RNA, and in situ hybridization.

Two blood draws of 10 ml will be part of the study. Baseline complete blood count including white blood cells and differential, liver function tests, and alpha 1-antitrypsin level will be obtained. Forty-eight hours after nasal instillation of the DNA/liposome mixture, the second blood draw will be done and the above chemistries will be measured and compared to baseline. On the second sample, DNA isolation will be performed and PCR done to detect the presence of plasmid in the circulating blood. In addition, baseline sinus radiographs and sinus radiographs at 48 hours after instillation of the DNA/liposome complex will be obtained and comparison of the two radiographs performed to evaluate for evidence of sinusitis.

Many patients with alpha 1-antitrypsin deficiency are receiving alpha 1-antitrypsin protein replacement therapy. If patients are receiving replacement therapy, they will be required to stop this one month prior to their entrance in this study to insure that alpha 1-antitrypsin measurements are due to expression of the exogenously delivered alpha 1-antitrypsin gene rather than replacement protein therapy. Cessation of replacement therapy will not exceed 6 weeks. Given the nature of the underlying disease, cessation of replacement protein therapy for no more than 6 weeks would not alter their overall clinical course. In addition, any nasal inhaler that the patient may be using will be stopped during the one week of the study to minimize possible washout of the gene. Again, temporary cessation of nasal inhalers should not adversely affect their patient's overall medical course.

Other Methods

Alpha-1 antitrypsin measurements In addition to immunohistochemical staining and western analysis, we will also measure AAT in blood, BAL fluid and extracts of tissues using an ELISA method employing a double antibody sandwich technique (26). The method is specific for the human protein.

Tissue and cell preparation Tissue or cells obtained by nasal scraping will be fixed according to their ultimate use. For routine light microscopy of lobes or lungs obtained at surgery, we will flush the pulmonary vasculature with phosphate buffered saline (PBS) prior to either airway inflation with 10% formol-saline from a pressure of 25cm H_2O or by intravenous injection, after tying the pulmonary veins at the hilum, from a pressure of 50cm H_2O. If only small tissue samples are obtainable at autopsy, then they will be immersion fixed for light microscopy. Small samples will be immersion fixed in glutaraldehyde for electron microscopic examination. Some samples will be infiltrated with OCT (Miles, Elkhart, IN) and rapidly frozen in liquid nitrogen. Tissue or cells for in situ hybridization will be fixed overnight in 4% paraformaldehyde in PBS (pH 7.2) at 4°C. Specific protocols for tissue preparation are included below.

Immunohistochemical staining Demonstration of AAT will be carried out on lung tissue or nasal epithelial cells which are fixed in 10% formol-saline. After 24 hours fixation at 4°C, several blocks will be taken and processed for routine light microscopy. Immunohistochemical localization will be carried out as previously described using either a rabbit or a sheep

polyclonal antibody to human AAT. (The Binding Site) and visualized with AP-Red (Zymed, San Francisco, CA).

In situ hybridization We will use an in situ hybridization technique similar to that published by Prosser et al. (27), Quagliano et al. (28) and Pelton et al. (29). Wherever possible lungs will be perfused free of blood and the inflated during fixation. Immediately on removal, tissue or cells are placed in freshly made 4% paraformaldehyde in PBS at 4°C for 12-24 hours. Tissue is then dehydrated through graded ethanol and xylene and embedded in paraffin wax. Five μm thick sections are cut, floated on DEPC distilled water, mounted on acid-washed, poly L-lysine coated glass slides and dried for 2 hours at 55°C. Sections are then deparaffinized in xylene, rehydrated through graded alcohols and left in PBS for 5 min. Sections are treated with proteinase K for 125 min at 37°C followed by treatment with 0.1M triethanolamine buffer, pH 8.0 for 5 min and then freshly prepared triethanolamine buffer containing 0.25% acetic anhydride. Slides are then washed twice in 2xSCC, dehydrated through alcohols and air dried.

We plan to use sense and antisense riboprobes (approximately 200-300 bases in length) generated from the desired cDNA. The sections will be prehybridized at 42°C for 1h and then hybridized with ^{35}S-labeled probe, incubated overnight in a humidified chamber at 42°C. After incubation, slides will be washed, dehydrated through alcohols, air dried, dipped in Kodak NTB-2 emulsion and stored at 4°C for 1-2 weeks. Slides will then be developed and stained and viewed with both bright and dark field illumination. Controls for the ^{35}S-labeled antisense riboprobes will include unrelated oligomers and sense riboprobes as appropriate.

Northern analyses Total cellular RNA will be isolated from tissues by standard methods (30). To detect RNA from the transfected gene, total RNA will be size fractionated in a formaldehyde/agarose gel and the blot transferred to a nitrocellulose membrane. The blot will be hybridized with a probe specific for human AAT, autoradiograms will be performed and quantitated by laser scanner.

Western analyses Total protein concentration in lavage fluid or tissue extracts will be determined by the Coomasie Blue method of Read and Northcote (31). Aliquots of the extracts will be subjected to polyacrylamide gel electrophoresis (PAGE) under denaturing conditions using standard methods (32). Following electrophoresis, the protein will be transferred electrically to a sheet of nitrocellulose in a Transblot chamber (Bio-Rad, Inc.) and immunodetection performed by published methods (33). Appropriate first and second antisera will be obtained from The Binding Site, San Diego, CA. We will use a second antiserum with an alkaline phosphatase conjugated group. The substrate cleaved by the alkaline phosphatase is 5-bromo-4-indoyl phosphate which yields a chromogenic product in the presence of nitroblue tetrazolium.

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CONSENT FOR RESEARCH STUDY

ADULT: (Patient) __X__ CHILDREN: (Patient) _____ PARENTS _____

(Volunteer) _____ (Volunteer) _____ GUARDIANS _____

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in the Lungs

NAME OF RESEARCH VOLUNTEER:_____ AGE:_____

TO PERSONS WHO AGREE TO PARTICIPATE IN THIS STUDY:

The following information is provided to inform you about the research project and your participation in it. Please read this form carefully. Any questions you may have about this study will be answered. Please feel free to ask any questions you may have about this study and/or about the information given below.

Written form _____ Oral form _____

ITEM #1: **Indicated below are the following:**
- **(a) The purpose of this study**
- **(b) The procedures to be followed**
- **(c) The approximate duration of this study**

We want to see whether we can put a gene into the lungs in a way that the gene will produce a protein that protects the lungs from injury. This has never been done in humans although we have shown that it works and is safe in animals. We have used several different animals to put a gene into the lungs. We have used mice, rats, rabbits, and sheep. In all the animals we have studied, no side effects or complications occurred due to putting the gene into them. The gene (or DNA) that we are going to put into your lung is called the alpha 1-antitrypsin gene. This gene normally exists in humans and helps protect the lungs from damage. In order for us to put this gene into your lungs, small pieces of DNA from viruses and bacteria are included. These additional pieces of DNA are just bits of DNA. They cannot cause a viral infection or a bacterial infection. There is no risk of you developing cancer from this DNA.

To get the gene material into the lung cells, we mix it with a solution of very small fat particles called liposomes. Various types of liposomes have been used to deliver drugs to humans. The kind of liposome that we are using have been used before to deliver DNA to individuals and no side effects or complications were seen.

We chose to ask you to participate in this study because you are going to have one of your lungs removed as part of your medical care. Twenty four to forty eight hours before your surgeons are to remove that lung, we want to do a brief bronchoscopy; a small tube will be passed through your nose and into a small part of the lung to be removed. Bronchoscopy is a routine medical procedure done daily at Vanderbilt and other medical institutions. The bronchoscope is a long flexible tube with a light source at one end and an eyepiece at the other end. The diameter of the tube is approximately the diameter of a normal sized writing pen. Bronchoscopy is simple and safe. First, your nose will be made numb as will the back of your throat with a type of medicine similar to the numbing medicine used by dentists. The bronchoscope will be passed through your nose into the back of your throat. When the bronchoscope is advanced to your voice box, more numbing medicine will be put through the bronchoscope to numb your larynx. Once your voice box is numb, the bronchoscope will be advanced through the voice box into your lung. Once the bronchoscope is in your lung, it will be placed securely into a area of your lung that does not contain the cancer but within a part of the lung that the surgeon plans to remove. After the bronchoscope is secure in place, sterile water will be rinsed into the lung and collected to be examined for various laboratory tests.

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PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in the Lungs

ITEM 1 (Continued):

After the lung is washed with sterile water, we will inject into that same area of the lung the DNA mixed with liposomes. The DNA which we are using makes a protein (alpha 1-antitrypsin) that protects the lungs. Alpha 1-antitrypsin is a normal protein found in the lungs of humans.

The amount of material, DNA/liposome mixture, which we will inject is small. We only want to see whether this method works in human lungs. After we instill the DNA/liposome mixture, the bronchoscope will remain in place for approximately 15 minutes to make sure that as much of the DNA/liposome mixture is taken up by the lung. The bronchoscope will then be removed. If any excess fluid is seen when the bronchoscope is removed, this will be suctioned out of the lung through the bronchoscope. Then, after you have your surgery and the lung is removed, we will take part of the lung which has been removed to test whether the gene we put in works.

We also want to draw a small amount of blood from a vein in your arm just before the bronchoscopy and again at the time of your surgery. The total amount of blood will be about 4 tablespoonfuls. We will also obtain an additional chest x-ray prior to your surgery to evaluate for the presence of pneumonia.

The costs of the bronchoscopy and all laboratory work including chest x-ray will be paid by the Department of Pulmonary and Critical Care Medicine.

ITEM #2: The parts of this study that are being done solely for this study are:

What we propose to do is entirely for the purpose of this study. We will in no way interfere with or alter your medical care.

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in the Lungs

ITEM #3: The discomforts, inconveniences and/or risks that can reasonably be expected are:

The bronchoscopy necessary to put the material in your lung will be like the one you had earlier to determine what was wrong with your lung, except much briefer in duration and no pieces of tissue or other samples will be taken. You will be given a mild sedative and your nose and airway will be numbed with medicine to make it as comfortable as possible. The bronchoscopy will be performed in the outpatient surgery department of The Vanderbilt Clinic. Bronchoscopies are routinely done in this location and the outpatient surgery department is fully equipped to handle bronchoscopies. The bronchoscopy itself should take no more than 45 minutes to perform. Following bronchoscopy, you will be observed in the outpatient recovery room for approximately 3 hours.

Bronchoscopy is a safe and simple procedure. Because no pieces of tissues will be taken, the risks involved are even less than most routine bronchoscopies. Your blood pressure, heart rate, and the oxygen in your blood will be monitored throughout the procedure. There is an very low risk of serious bleeding when a bronchoscopy is done. We screen for bleeding abnormalities and because no pieces of tissues are taken, bleeding complications are unlikely. In your case, this risk will be less than 0.1%. Some individuals may experience a fall in the amount of oxygen in their blood. Again this is unlikely. We will monitor the oxygen in your blood and give extra oxygen as necessary. Occasionally, a collapsed lung occurs following bronchoscopy. This event is almost universally associated with obtaining pieces of tissue which we are not going to do. Other less common complications include pneumonia following bronchoscopy and irregular heart rhythm. Rarely, it is possible that the discomfort of the procedure or one of the drugs given will cause a reaction. Coughing is associated with bronchoscopy. Whenever coughing is encountered we administer medicine to stop the cough.

The possibility of any complication occurring from the bronchoscopy is less than 1%.

There is no reason to believe that the DNA/liposome mixture should cause any problems for you. There is no risk that the DNA itself or the liposomes would cause any kind of infection or cancer. We know of no side effects of putting DNA or liposomes into your lungs. Even though the material will be put into the lung which will be removed, it is possible that some of the DNA or liposomes will be absorbed and go to some other organ like the liver. Should this occur, we have no reason to believe that it would cause any side effects.

If you are a woman of reproductive age, a pregnancy test will be required to document that you are not pregnant. In addition, contraception is required for one month prior to the study and for two months following the study. This is strictly a safety precaution. Studies in animals given DNA/liposome mixtures have not detected any of the delivered DNA in reproductive organs.

The possibility that this bronchoscopy will result in a delay in your surgery is extremely low, less than the possiblity of a complication from the bronchoscopy. Our study will be conducted in conjunction with your surgeon and will be coordinated according to what is the best schedule for you. If, by some very unusual circumstances, the bronchoscopy should delay your surgery, a short delay would not alter the overall outcome or prognosis of your medical condition.

NOTE: Immediate necessary care will be provided without charge by Vanderbilt University if you are injured because of participation in this research project. However, there is no provision for the costs of further medical care or for the monetary compensation for such injury.

ITEM #4: The possible benefits to you from this study are:

You will receive no direct benefit from this study.

ITEM #5: The benefits to science and to mankind that are sought by this study are:

The possibility of gene therapy is an entirely new area which may be beneficial in the large number of human diseases. This study is a first step in that direction and, if successful, will pave the way for application of this method in patients with lung diseases like emphysema.

ITEM #6:(a) Alternate procedures that might be advantageous; but which will not be used are:

There are no alternate procedures.

(b) The reasons for not using them are:

N/A

ITEM #7: Your rights of privacy will be maintained in the following manner:

(1)All data obtained on you during the course of this study will be kept confidential and accessible only to the principal investigator and his/her assistants on this project; except as stated elsewhere.

(2)Should the results of this project be published, you will be referred to only by number.

NOTE:YOU ARE FREE TO WITHDRAW THIS CONSENT AND TO DISCONTINUE PARTICIPATION IN THIS STUDY OR ACTIVITY AT ANY TIME.

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in the Lungs

STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS RESEARCH PROJECT:

_____(a)I have read this Consent Form. All my questions have been answered and I freely and voluntarily choose to participate. I understand that I may withdraw at any time. I understand that my rights and privacy will be maintained. I also understand that in the event this study is being funded through the Department of Health and Human Services (DHHS), The National Institutes of Health (NIH)...the Secretary of the Department of Health and Human Services or his designated representatives (such as the Food and Drug Adminstration) does have free access to any information obtained in the study should it become necessary. I also understand that when a research study involves the use of drugs and the study and/or the drugs are sponsored by any "independent organization" (that is, not sponsored by the Department of Health and Human Services or any other federal agency), the records of that study are accessible to the study/drug sponsors udner the conditions outlined in the Food and Drug Administration (FDA) Guidelines. I also understand that in such independently sponsored studies, the FDA also has access to these records should it become necessary.

I agree to become a participant in this program.

_____(b)The material contained in this Consent Form has been explained to me orally. All my questions have been answered and I freely and voluntarily choose to participate. I understand that I may withdraw at any time. I understand that my rights and privacy will be maintained. I also understand that in the event this study is being funded through the Department of Health and Human Services (DHHS), The National Institutes of Health (NIH)...the Secretary of the Department of Health and Human Services or his designated representative (such as the Food and Drug Administration) does have free access to any information obtained in the study should it become necessary. I also understand that when a research study involves the use of drugs and the study and/or the drugs are sponsored by an "independent organization" (that is, not sponsored by the Department of Health and Human Services or any other federal agency), the records of that study are accessible to the study/drug sponsors under the conditions outlined in the Food and Drug Administration (FDA) Guidelines. I also understand that in such independently sponsored studies, the FDA also has access to these records should it become necessary.

I agree to become a participate in this program.

(Date) Signature of Participant

(Date) Signature of Witness(es), when applicable

CONSENT FOR RESEARCH STUDY

ADULT: (Patient) __X__ CHILDREN: (Patient) _____ PARENTS _____

(Volunteer) _____ (Volunteer) _____ GUARDIANS _____

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in Nasal Epithelium

NAME OF RESEARCH VOLUNTEER:_____ AGE:_____

TO PERSONS WHO AGREE TO PARTICIPATE IN THIS STUDY:

The following information is provided to inform you about the research project and your participation in it. Please read this form carefully. Any questions you may have about this study will be answered. Please feel free to ask any questions you may have about this study and/or about the information given below.

Written form _____ Oral form _____

ITEM #1: **Indicated below are the following:**
- (a) **The purpose of this study**
- (b) **The procedures to be followed**
- (c) **The approximate duration of this study**

We want to see whether we can put the alpha 1-antitrypsin gene into the cells of the nose of individuals with alpha 1-antitrypsin deficiency in a way that the gene will produce the alpha 1-antitrypsin protein. This has never been done in humans although we have shown that we can put genes into other animals and that the gene works and is safe. We have put genes into mice, rats, rabbits, and sheep. In all the animals we have studied, no side effects or complications occurred due to putting the gene into them. The alpha 1-antitrypsin gene normally exists in humans and helps protect the lungs from damage. In order for us to put this gene into your nose, small pieces of DNA from viruses and bacteria are included. These additional pieces of DNA are just bits of DNA. They cannot cause a viral infection or a bacterial infection. There is no risk of you developing cancer from this DNA.

To get the gene material into the nose cells, we mix it with a solution of very small fat particles called liposomes. Various types of liposomes have been used to deliver drugs to humans. The kind of liposome that we are using have been used before to deliver DNA to individuals and no side effects or complications were seen.

We chose to ask you to participate in this study because you have alpha 1-antitrypsin deficiency. In addition, because you do not normally produce alpha 1-antitrypsin, we will be able to accurately measure the amount of protein that is produced following introduction of the alpha 1-antitrypsin gene into your nose. Although the absence of alpha 1-antitrypsin causes lung disease, not nasal disease, this technique allows a simple manner to assess how well our method of gene therapy works.

Baseline alpha 1-antitrypsin level in your nasal washing will be obtained. This involves washing your nose with less than a teaspoon of salt water. The nasal wash is simple to perform. The salt water will be drawn up into a syringe and a small tubing will be attached to the end of the syringe. The free end of the tubing will be inserted approximately one inch into one side of your nose. After the tubing is placed in your nose, you will place your chin to your chest, block your nose with either your free hand or with nose clips, and infuse the salt water into your nose. After infusion, the salt water will be collected into a tube by gravity drainage.

After we establish your baseline alpha 1-antitrypsin level in the nasal washing, we will infuse some DNA mixed with liposomes into your nose. The amount of material which we will infuse is small. We only want to see whether this method works in nasal cells. This entire procedure should require no more than thirty minutes.

After the gene is put in your nose, we will obtain nasal washings and nasal scrapings three days, five days, and seven days after we have given you the gene. To obtain nasal scrapings, we use a small sterile instrument that gently removes some of the cells from the lining of your nose. Nasal scrapings will allow us to obtain further documentation that the gene is working.

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PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in Nasal Epithelium

ITEM #1 (Continued):

Two other laboratory tests are also part of this study. Before we put the gene in your nose, we will obtain a small amount of blood from a vein in your arm to measure various laboratory values. 48 hours after we put the gene in your nose, we will repeat these same laboratory tests on another blood sample and compare these laboratory values to your baseline. The total amount of blood will be about 4 tablespoonfuls. The other laboratory tests we will do are x-rays of your sinuses. The sinus x-rays are additional x-rays which you normally would not receive. The additional amount of radiation that you will receive from one 4 film skull series (sinus x-rays) is about the same as six weeks of natural background radiation. We will obtain a baseline x-ray of your sinuses before we put the gene in and then 48 hours afterwards to evaluate for the presence or absence of sinusitis. Both the blood draws and sinus x-rays are routine medical tests that are done daily. The costs for the laboratory work and x-rays will be covered by the Department of Pulmonary and Critical Care Medicine.

If you are on alpha 1-antitrypsin protein replacement therapy (Prolastin), you will have to stop this one month before this study to insure that the alpha 1-antitrypsin values that we obtain during the study are due to the gene that we put in your nose and not from the Prolastin. Also, if you are on any nasal inhalers, these will need to be stopped during the study (for one week).

ITEM #2: The parts of this study that are being done solely for this study are:

What we propose to do is entirely for the purpose of this study.

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in Nasal Epithelium

ITEM #3: The discomforts, inconveniences and/or risks that can reasonably be expected are:

The procedure used to put the alpha 1-antitrypsin gene into your nose may cause a temporary sense of irritation in your nose and it may cause you to sneeze. Some persons experience increase tearing from their eyes for a brief period; this is also temporary. Although unlikely, you may develop sinusitis following administration of the material into your nose. If this should happen, routine medical care for sinusitis will be administered. We know of no side effects of putting DNA or liposomes into your nose. It is possible that some of the DNA material will be absorbed and go to some other organ like the liver. Should this occur, we have no reason to believe that it would be harmful. Given the nature of alpha 1-antitrypsin deficiency, temporarily stopping your alpha 1-antitrypsin replacement therapy or your nasal sprays should in no way affect your long term medical care or course.

Pregnant women are excluded from this study. If you are a woman of reproductive age, a pregnancy test will be performed to document that you are not pregnant. If you are not pregnant and choose to participate in this study, contraception is required for one month prior to the study and for two months following the study. This is strictly a safety precaution. Studies in animals given DNA/liposome mixtures have not detected any of the delivered DNA in reproductive organs.

NOTE: Immediate necessary care will be provided without charge by Vanderbilt University if you are injured because of participation in this research project. However, there is no provision for the costs of further medical care or for the monetary compensation for such injury.

ITEM #4: The possible benefits to you from this study are:

None

ITEM #5: The benefits to science and to mankind that are sought by this study are:

The possibility of gene therapy is an entirely new area which may be beneficial in a large number of human diseases. This study is a first step in that direction and, if successful, will pave the way for application of this method in patients with lung diseases like emphysema.

ITEM #6:(a) Alternate procedures that might be advantageous; but which will not be used are:

There are no alternate procedures.

(b) The reasons for not using them are:

N/A

ITEM #7: Your rights of privacy will be maintained in the following manner:

(1)All data obtained on you during the course of this study will be kept confidential and accessible only to the principal investigator and his/her assistants on this project; except as stated elsewhere.

(2)Should the results of this project be published, you will be referred to only by number.

NOTE:YOU ARE FREE TO WITHDRAW THIS CONSENT AND TO DISCONTINUE PARTICIPATION IN THIS STUDY OR ACTIVITY AT ANY TIME.

PRINCIPAL INVESTIGATOR: Kenneth L. Brigham, M.D.
TITLE OF PROPOSAL: Expression of an Exogenously Delivered Human Alpha-1 Antitrypsin Gene in Nasal Epithelium

STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS RESEARCH PROJECT:

_____(a)I have read this Consent Form. All my questions have been answered and I freely and voluntarily choose to participate. I understand that I may withdraw at any time. I understand that my rights and privacy will be maintained. I also understand that in the event this study is being funded through the Department of Health and Human Services (DHHS), The National Institutes of Health (NIH)...the Secretary of the Department of Health and Human Services or his designated representatives (such as the Food and Drug Adminstration) does have free access to any information obtained in the study should it become necessary. I also understand that when a research study involves the use of drugs and the study and/or the drugs are sponsored by any "independent organization" (that is, not sponsored by the Department of Health and Human Services or any other federal agency), the records of that study are accessible to the study/drug sponsors udner the conditions outlined in the Food and Drug Administration (FDA) Guidelines. I also understand that in such independently sponsored studies, the FDA also has access to these records should it become necessary.

I agree to become a participant in this program.

_____(b)The material contained in this Consent Form has been explained to me orally. All my questions have been answered and I freely and voluntarily choose to participate. I understand that I may withdraw at any time. I understand that my rights and privacy will be maintained. I also understand that in the event this study is being funded through the Department of Health and Human Services (DHHS), The National Institutes of Health (NIH)...the Secretary of the Department of Health and Human Services or his designated representative (such as the Food and Drug Administration) does have free access to any information obtained in the study should it become necessary. I also understand that when a research study involves the use of drugs and the study and/or the drugs are sponsored by an "independent organization" (that is, not sponsored by the Department of Health and Human Services or any other federal agency), the records of that study are accessible to the study/drug sponsors under the conditions outlined in the Food and Drug Administration (FDA) Guidelines. I also understand that in such independently sponsored studies, the FDA also has access to these records should it become necessary.

I agree to become a participate in this program.

(Date) Signature of Participant

(Date) Signature of Witness(es), when applicable

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person

CVs

NAME	POSITION TITLE
Brigham, Kenneth L.	Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
David Lipscomb College Nashville, Tennessee	B.A.	1962	Biology
Vanderbilt University Nashville, Tennessee	M.D.	1966	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided that they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Research and Professional Experience:

1966-1967	Intern, Osler Medical Service, Johns Hopkins Hospital, Baltimore, Maryland
1967-1968	Assistant Resident in Medicine, Osler Medical Service, Johns Hopkins Hospital, Baltimore, Maryland
1968	Cholera Research Unit, Johns Hopkins Center for Medical Research and Training, Calcutta, India (spent three months of assistant residency year)
1968-1970	Medical Epidemiologist, U.S. Public Health Service, National Communicable Disease Center, Ecological Investigations Program, Phoenix Laboratories, Phoenix, Arizona
1970-1971	Instructor in Medicine and Fellow in Pulmonary Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee
1971-1973	Research Fellow, Cardiovascular Research Institute, University of California Medical Center, San Francisco, California
1973-1976	Director of Pulmonary Research, Vanderbilt University School of Medicine, Nashville, Tennessee
1973-1974	Assistant Professor of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1974-1978	Associate Professor of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1976-Pres.	Director, Center for Lung Research (Specialized Center of Research in Pulmonary Vascular Diseases), Vanderbilt University School of Medicine, Nashville, Tennessee
1977-1986	Associate Professor of Biomedical Engineering, Vanderbilt University School of Medicine, Nashville, Tennessee
1978-Pres.	Professor of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1978-Pres.	Director, Division of Pulmonary Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1983-1985	Assistant Professor of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee
1984-Pres.	Joe & Morris Werthan Professor of Investigative Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1985-Pres.	Associate Professor of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee
1986-Pres.	Professor of Biomedical Engineering, Vanderbilt University School of Medicine, Nashville, Tennessee

Research and Professional Experience (Continued)

1988 Visiting Scientist, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee (January through June, 1988)

Publications (Selected):

1. Brigham, K.L., Stahlman, M.T., Eds. Respiratory Distress Syndrome: Molecules to Man. Vanderbilt Univ. Press, Nashville, TN, 1990.
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HUMAN GENE TRANSFER PROTOCOL

9403-071 Vogelzang, Nicholas; the University of Chicago, Chicago, Illinois; *Phase I Study of Immunotherapy for Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions.*

Date of RAC Approval: 3/4/1994
Date of NIH Approval: 4/19/1994

Scientific Abstract

The goal of immunotherapy is to stimulate the immune system by modification of tumor cells or expansion of lymphocytes which respond specifically to tumor antigens. In this study, we will apply techniques of direct gene transfer to enhance immune response against tumors *in vivo*. Patients with advanced cancer who have failed all effective therapy will be treated by injection of a DNA/lipid complex directly within the tumor. DNA will be used which encodes a heterodimeric cell surface protein recognized in the transplantation response. These genes include the HLA-B7 histocompatibility antigen and β-2 microglobulin gene in a non-viral plasmid eukaryotic expression vector. For this vector, a safe and effective dose to introduce this recombinant gene in HLA-B7 negative patients will be established. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. We will also determine whether this treatment facilitates tumor regression. This study employs the same study drug as Dr. Gary Nabel of the University of Michigan, previously proposed gene therapy protocol, but expands the study to additional clinical sites, the Mayo Clinic, the Arizona Cancer Center, and the University of Chicago Medical Center. These studies will facilitate the development of other approaches, using different recombinant genes or in combination with cytokines or adoptive T-cell therapy, to augment tumor immunity, and allow for greater potential efficacy. This method will also establish the safety of this non-viral approach to gene therapy, which could potentially be extended to treat a variety of other human diseases.

Non-technical Abstract

Many types of cancer cannot be cured by traditional medical treatrnents, including drugs, surgery, or radiation. In this study, an experimental treatment will be offered that may help to fight this disease. We will attempt to induce tumor regression by the introduction of genetic material that directs the synthesis of proteins which stimulate the immune system. The genetic material, DNA, will be introduced directly into the tumor by mixing it with fatty substances, or lipids, and this mixture will be injected into the tumor. The DNA will be taken into cells and cause them to produce proteins that stimulate tissue rejection. These proteins, called histocompatibility proteins, cause cells which contain it to be recognized as foreign by the immune system. The goal of the treatment is to stimulate the immune system to attack and kill the tumor. In this study, we will attempt to determine the optimal safe and effective dose to administer the DNA/lipid complex. Increasing amounts of this complex will be used in different patient populations. If no side effects are observed, repeated treatments may be instituted. The expression and nature of the immune response will also be characterized. This treatment may provide a therapeutic effect in cancer and could be applied to the treatment of other diseases.

Phase I Study of Immunotherapy of Metastatic Renal Cell Carcinoma by Direct Gene Transfer Into Metastasic Lesions

Protocol

(This protocol is identical in background, goals, patient monitoring, and drug preparation and supply as the master protocol "Phase I/II Trial of HLA-B7 Plasmid DNA/DMRIE/DOPE Complex as an Immunotherapeutic Agent in Solid Malignant tumors by Direct Gene Transfer." In addition, it contains the details of patient eligibility and treatment specific to a study of metastatic renal cell carcinoma proposed by and to be carried out at the University of Chicago.)

Principal Investigator:	Nicholas J. Vogelzang, M.D.
Co-Investigators:	Timothy M. Lestingi, M.D. Gary Sudakoff, M.D.
Sponsor:	Vical Inc.
Date:	December 20, 1993

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Schema

Prior to discussing protocol entry with the patient, call the Research nurse to insure that a place on the protocol is open to the patient. Escalating doses will be evaluated. Groups of patients will receive either escalating doses of an intralesional injection of the test agent into one metastatic lesion in a single session (schedule A) or repeated injections at the same dose (schedule B).

Schedule A

Group	Number of Patients	mcg of DNA
A-1	3	10 mcg day 1 only
A-2	3	50 mcg day 1 only
A-3	3	250 mcg day 1 only

Schedule B
(Will be initiated after safety of Schedule A at 50mcg has been established)

Group	Number of Patients	mcg of DNA
B-1	3	10mcg day 1 and 15
B-2	3	10mcg day 1, 15 and 30

Schedule A

A 1 to 3 REG	Rx (day 1)	4 wks Re-evaluation		PR may be retreated once < PR Observation

Schedule B

B-1 REG	Rx (day 1)	Rx (day 15)		4 wk Re-evaluation	PR - cycle of 2 injections 15 days apart may be repeated once < PR no Rx Observation
B-2 REG	Rx (day 1)	Rx (day 15)	Rx (day 30)	2 wk Re-evaluation	PR - cycle of 3 injections each 15 days apart may be repeated once < PR - no Rx Observation

1.0 Background

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells, are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5-10% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor

cells with high efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to modulation of immune function, because the immune system can be induced to recognize tumor associated and tumor specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have given about a 10-20% response rate. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive transfer of cytotoxic T cells (CTL's), which have shown promise in animal models (1-6) and in man (7-10). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy.

Nabel and colleagues at the University of Michigan are investigating a novel molecular genetic intervention for human malignancy that enhances the immune response to tumors by in vivo gene transfer. This immunotherapeutic approach based on animal model work (11, 12) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7, introduced into human tumors in vivo by DNA/lipid complex transfection. The direct intratumoral injection approach is used. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells. The introduction of an allogeneic MHC gene directly into tumors in vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens.

In a preliminary trial in humans with malignant melanoma Nabel treated 5 patients with malignant melanoma. Three patients received 3 treatments, totaling 0.87 μg of DNA intratumorally, and 3 patients (2 additional patients plus one of the original 3 patients) received cumulative dose of 2.58 μg of DNA via three treatments. No toxicity resulted from this form of treatment and there was no formation of anti DNA antibody or autoantibody. There was no plasmid DNA detectable in the blood by PCR following gene transfer (tested on days 3-7 post transfection at ~2 pg/ml sensitivity).

Evidence of gene transfer was found on biopsy of the injected tumor. The biopsy samples were analyzed for plasmid DNA, mRNA coding for HLA-B7 and the expression of HLA-B7 protein. In 4 of the 5 patients, plasmid DNA and HLA-B7 mRNA were detected within the treated nodules by PCR. HLA-B7 expression was confirmed in all treated nodules by immunohistochemical staining with a monoclonal antibody to the gene product. Two

patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells. One of the 5 patients had a partial remission which involved cutaneous and visceral disease. (Nabel, et al., PNAS, 1993).

These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

Several improvements that may increase the convenience, safety and efficacy of the procedure have been introduced since the original Nabel studies including:

- an improved cationic lipid formulation, DMRIE/DOPE;
- DNA plasmid construction to optimize expression

The efficacy of transfection was improved for the following reasons. Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner (Vical) in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency in vitro compared to the formulation previously used by Nabel, et al. More importantly, this formulation does not aggregate at high concentrations. This characteristic thus allows higher absolute concentrations of DNA and lipid complex to be introduced into experimental animals without toxicity. Because of these properties, it now becomes possible to introduce 100-1000 times more DNA which could allow the study of an expanded dose response gene expression in vivo.

The vector improvements are divided into two categories for this proposal. In the first case expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the ß-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface. This is important because some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. It has been found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. These modifications have been incorporated in the study drug to be used in this submission. The study drug is identical to the study drug fully characterized in Dr. Nabel's RAC submission of June 7, 1993, which was unanimously allowed. This study will investigate the administration of the study drug in metastatic renal cell cancer.

1.2 Background

1.2.1 Direct Gene Transfer and Modulation ofthe Immune System

The utilization of catheter-based gene delivery in vivo provided a model system for the introduction of recombinant gene-specific sites in vivo. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (13,14). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA lipid complex (12). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site in vivo. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene in vivo (12).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product in vivo. In addition, this approach provided a general method for the induction of a specific site in vivo. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy was developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (11). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.2.2 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (15,16) and lymphokine-activated killer (LAK) cells (17). Cytolytic T cells which infiltrate tumors have been isolated and characterized (18). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (3,19). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (20,21), or by activation induced by substances such as BCG (22).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell

growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-a (TNF-a) (23) and lymphotoxin (24) can result in neoplastic cell death. Interferon- (IFN-_) markedly increases class I MHC cell surface expression (25,26) and synergizes with TNF-_ in producing this effect (27). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (28), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (17,29,30). The LAK cells lyse tumor cells without preimmunization or MHC restriction (31). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (32).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanism to escape host defenses, perhaps through evolution of successively less immunogenic clones (33). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (34-38). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (39-43). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (40,41). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (44).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (45), or IL-2 with LAK cells (46,47). Clinical trials using tumor infiltrating lymphocytes are also in progress (48). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (49). Expression of IL-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (50). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

Nabel and co-workers have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (51-55), or expression of allogeneic antigens introduced by somatic cell hybridization (56,57). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection in vitro. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene (H-2L^{d}) into an H-2^{b} tumor resulted in

immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (58). In another instance, transfection of H-2^b melanoma cells with the H-$2D^d$ gene did not lead to rejection (59), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (60). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (61). Several subclones which were selected in vitro and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggests that clone-to-clone variation in in vivo growth and tumorigenic capacity may result in other modifications of cells caused by transfection or the subcloning procedure, which affects their tumorgenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly in vivo.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in our animal model studies designed to enhance the immunogenicity of tumors in vivo. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by in vivo infection using retroviral vectors or by DNA lipid complex mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection in vivo.

1.2.3 Intra-lesional Therapy

The Department of Diagnostic Radiology at the University of Chicago has extensive experience in CT and sonographically-guided needle biopsy. Sonographically-- guided biopsy of metastatic lesions can be carried out with a high degree of accuracy and safety. It is almost always possible to obtain a core rather than an aspirate of tissue by using a 16-19 gauge needle, needles with cutting ends, and improved biopsy sampling technology. In a review from the Mayo Clinic, overall accuracy was 91% in the biopsy of 126 consecutive masses of various histologic types 3cm or less in diameter. It is almost always possible to obtain a core rather than an aspirate of tissue by using a 16-19 guage needle, needles with cutting ends, and improved biopsy sampling technology. In a study from the May Clinic of 1,000 consecutive CT-guided biopsies, the rate of complication from the use of an 18-guage biopsy needle was 0.3%, the same as the rate of complication from the use of a 21-guage needle. This capability allows, in one sitting, biopsy of the intended target for gene transfer, documentation of metastatic cancer in the lesion, and delivery of the DNA/liposomal mixture to the targeted nodule(s). For purposes of this protocol, nodules 2-4 cm in size that are easily accessible will be selected to further ensure successful target injection and to reduce the possibility of morbidity.

One advantageous feature of sonographically-guided delivery of DNA/liposomal complex is that the distribution of the fluid within the injected area can be visualized and documented on videotape. Apparently, the release of small bubbles from dissolved air in fluids at room temperature injected into tissue causes the area perfused to become hyper echoic. This allows the radiologist to observe the regions of the nodule being injected.

Excellent facilities exist at the University of Chicago for carrying out sonographically-directed biopsy and delivery of materials at deep sites in the body. For this in vivo gene transfer study, patients will be admitted to the Hematology/Oncology service at the University of Chicago Hospital. Invasive procedures in the ultrasound suite are carried out under conditions in which a general surgeon is immediately available in the extremely rare instance of the acute complication of bleeding. An operating room is always immediately available for such circumstances. A CT scanner will also be available to visualize localized bleeding. Following the procedure, the patient will be kept in the diagnostic radiology area until stable. The patient will then be transferred to the inpatient Hematology/Oncology ward.

The chemotherapy, pharmacy, and pharmacology shared resources of the University of Chicago Cancer Center are available and will support this project. The pharmacy will assure the storage, preparation, and delivery of the final DNA/liposomal product to the ultrasound suite when needed.

The design of this trial is similar to standard phase I clinical trials. HLA-B7 negative patients with metastatic renal cell carcinoma will be enrolled. Patients must have at least 2 metastatic lesions or one metastatic lesion and an intact primary which are clearly measurable by CT scan and one of which is appropriate for sonographically-guided biopsy and injection. Patients will be treated on one of two schedules. On schedule A, the recombinant DNA will be administered in escalating doses to groups of 3 patients at each of 3 dose levels: 10, 50, and 250 mcg. On schedule A, each patient will receive only a single injection. Each of 3 patients will be observed for toxicity and response for 30 days before patients will receive DNA at the next planned higher dose, 50 mcg. The same procedure will be followed at this dose level before proceeding to the final dose level, 250 mcg. Schedule B will be initiated in new patients after all 3 patients given 50 mcg DNA have been observed and found not to have unacceptable toxicity. On schedule B-1, a group of 3 patients will receive 10 mcg DNA days 1 and 15. If at 30 days no patient has unacceptable toxicity, a second group of 3 new patients, group B-2, will receive 10 mcg DNA on days 1, 15, and 30.

2.0 Goals

- 2.1 To determine safety and toxicity of direct intralesional injection of increasing amounts of a DNA/lipid mixture: VCL-1005 (HLA-B7/DMRIE/DOPE) into tumors in selected patients with metastatic renal cell carcinoma. Escalating treatment regimens will be used and tumor growth evaluated.
- 2.2 To measure the cytotoxic T-cell activity directed towards antigens on tumor cells other than HLA-B7.
- 2.3 To measure humoral and cellular immune responses to HLA-B7.
- 2.4 To confirm expression in vivo of the HLA-B7 gene in the tumor cells.
- 2.5 To characterize the clinical response to escalating doses of the study drug by assessing the size of the injected tumor and of other tumor masses that may be present.

3.0 Patient Eligibility

3.1 Inclusion criteria

Prior to discussing protocol entry with the patient, call the Research nurse (Diana Karius, R.N., M.S.N., 702-2085 or pager 6732) to insure that a place on the protocol is open to the patient.

3.1.1 Histologically-confirmed metastatic renal cell carcinoma.

3.1.2 At least two metastatic lesions or one metastatic lesion and an intact primary that are clearly measurable in two dimensions on CT scan. At least one metastasis must measure between 2.0 and 4.0 cm in greatest diameter and be accessible for intralesional injection.

3.1.3 Patients must have had prior standard therapy for their disease and have become unresponsive to it or have made the decision that other therapy would not be of any benefit.

3.1.4 Must be 18 years old.

3.1.5 The following laboratory values within 15 days of registration:
white blood count >3,000.
platelet count >100,000.
Hemoglobin >9g/dl.
prothrombin time < 1.5 x control.
serum creatinine < 2.0 mg/dL.
total bilirubin $\leq$ 1.5 unless due to Gilbert's disease.
SGOT, SGPT < 3x the upper limit of normal unless due to disease

3.1.6 Karnofsky Performance Scale (KPS) score $\geq$ 70.

3.1.7 Estimated life expectancy of at least 12 weeks.

3.1.8 HLA typing must indicate that the patient is HLA-B7 negative.

3.1.9 Must be anti-HIV antibody negative.

3.1.10 Women/men of reproductive potential agree to use effective contraception.

3.1.11 Patients must be immunocompetent by antigen skin test.

3.2 Exclusion criteria

3.2.1 Positive antibody to HIV.

3.2.2 Active autoimmune disease.

3.2.3 Hepatitis (acute or chronic active).

3.2.4 Active infection.

3.2.5 No radiation, biologic therapy or chemotherapy with 4 weeks (6 weeks for mitomycin of nitrosourea).

3.2.6 No corticosteroids within 2 weeks.

3.2.7 Diabetes mellitus not controlled by medical treatment.

3.2.8 Psychiatric illness that may make compliance to the clinical protocol unmanageable or may compromise the patient's ability to give informed consent.

4.0 Test Schedule

Tests and Procedures	<15 days prior to regis-tration	During course of Rx days 3, 15, and 29[7]	Follow-up: at 8 wks post Rx and q 8 wks until PROG, then q 8-12 wks
History and exam, tumor meas., ht, wt, KPS	X	X	X[10]
Vital signs (pulse, blood pressure, temperature)		X[1]	
WBC, HgB, PLT	X	X	X
Chem-17, SGPT	X	X	X
PT, PTT	X	Days 15 and 29 only	Day 57[7]
EKG	X		
Chest x-ray	X		
CT scan and/or ultrasound	X[8]	Days 15 and 29 only	X
Tumor biopsy, guided by ultrasound		Days 15 and 29 only	Days 43[7] and 57[7]
Urinalysis	X		
HbAg, HIV titer	X		
HLA-B7 phenotype	X[9]		
Assay for cytoxic T-cells and antibody[2]		X	X
Peripheral blood sample	X[3]	X[4]	Day 57[7]
Antinuclear antibodies	X	Day 29 only	Day 57[7]
Serum preg. test[6]	X		

1. Prior to each biopsy and treatment and then every 15 minutes until stable.
2. Biopsy tissue will be assayed for the presence of the HLA-B7 gene by PCR. Expression of the HLA-B7 gene will be looked for immunohistochemically. When sufficient materials are present, attempts to assess the development of cytolytic T-cell response to the cells of the tumor will be evaluated. Tissue will be "snap" frozen in liquid nitrogen (LN) and sent in LN to a central lab facility.
3. 45ml of clotted blood for serum storage and 45ml of anti coagulated blood for mononuclear cell cryopreservation will be obtained and sent to a central lab facility.
4. 20ml of clotted blood will be obtained to look for development of antibodies, and 20ml of anti coagulated blood for mononuclear cell cryopreservation will be obtained and sent to a central lab facility.
5. Only for patients receiving 2 or 3 injections of DNA on schedule B.
6. For women of childbearing potential only. Must be done within 7 days prior to registration.
7. Days 29, 43, and 57 are approximate. The goal will be to obtain the

studies as close as possible to 4, 6, and 8 weeks +/- 2 days.

8. Baseline CT scan must be done within 4 weeks of treatment.
9. At any time prior to treatment.
10. Survival follow-up will be life-long.

5.0 Stratification Factors

None.

6.0 Registration/Randomization Procedure

6.1 Prior to discussing protocol entry with the patient, call the Research nurse to insure that a place on the protocol is open to the patient.

6.2 To register a patient, call Scott McKay (702-2084) in the Data management office.

6.3 A signed consent form must be on file in the data management office before a patient may be registered.

6.4 Patient eligibility and the existence of a signed consent form will be checked by data management personnel before a patient will be registered into this study. Dose level will be given.

6.5 Treatment on this protocol must be given at the University of Chicago on an inpatient basis under the supervision of an attending physician in Hematology/Oncology. Actual intralesional injection of tumor will be carried out by Dr. Gary Sudakoff or one of his associates in the Department of Diagnostic Radiology.

6.6 Treatment must begin within 15 days of registration.

6.7 Pre treatment tests must be completed within the guidelines specified on the test schedule.

7.0 Drug Information/Protocol Treatment

7.1 The study drug (VCL-1005) will be supplied by Vical as two sterile vials containing (i) HLA-B7 plasmid DNA, and (ii) DMRIE/DOPE lipid mixture. Diluent will be lactated Ringer's. All components will be stable for at least eight weeks under recommended storage conditions (DNA -20°C, DMRIE/DOPE 4°C). The materials will be supplied by Vical Inc.

This study drug is composed of plasmid DNA coding for the complete human MHC HLA-B7 formulated with the cationic lipid mixture DMRIE/DOPE (lipid complex formulation). The DNA concentration is 1.0 mg/ml (see Investigator's brochure for complete details of product characteristics and preparation).

Lactated Ringer's is readily available at the site.

7.2 Identification and localization of a specific metastasis for injection using ultrasound (or CT scan if not visible by ultrasound) meeting the criteria stated in section 3.0 will

be carried out by Dr. Gary Sudakoff, or one of his associates, prior to admission. Tumor lesions will be selected for treatment if they are accessible to intratumor administration by direct needle injection or intravascular catheter. These metastatic lesions will be located at any accessible site such as skin, lymph nodes, lung, liver, kidney or spleen for renal cell carcinoma. If necessary, the study drug will be injected with the aid of sonographic visualization of the metastasis.

Once all pretherapy tests and procedures are completed, patients will be admitted to the Hematology/Oncology service at the University of Chicago prior to each intralesional injection.

7.3 DNA/lipid complexes are prepared immediately prior to administration. DNA is supplied in 1.0 mg/ml concentration in 400 µl lactated Ringer's solution. Lipid (DMRIE/DOPE) is supplied as a dried film. Each vial contains 77 µg DMRIE and 90 µg DOPE. Each vial is reconstituted with 400 µl lactated Ringer's solutions by vortexing until homogeneous. The contents of the lipid vial is transferred into the DNA vial and mixed well by repeated inversion. The final concentration of the HLA-B7 plasmid DNA is 500 µg/ml. The amount of DNA injected into each tumor will range from 10µgm to 250 µg. Lower doses, 10 and 50 µg, will be prepared in a similar fashion or formulated as dilutions with lactated Ringer's.

7.4 The appropriate volume of DNA/lipid mixture prepared in the University of Chicago Pharmacy will be injected into the pre-selected metastasis on day 1. The injection is done by the diagnostic radiologist using standard procedures in the Diagnostic Radiology Suite. The needle will be inserted under direct sonographic (or CT) visualization. A variable volume of lipid preparation (up to 4ml) will be used but all DNA concentrations will be kept constant per dose level.

Prior to injection and following placement of the needle, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Up to 4ml of the DNA/lipid mixture will be injected as described above. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80mm Hg, the injection will be terminated immediately, and the patient will be closely monitored until blood pressure is normalized.

Immediately after the injection, a blood sample will be obtained to check serum enzymes, blood chemistries and cell counts, and to analyze by PCR for the presence of HLA-B7 Plasmid DNA in the peripheral blood. Every patient will be observed for 23 hours and another blood collection will be drawn. If there are no complications, the patient will be discharged. If any abnormalities appear, the patient will be closely observed. All toxicities will be graded according to the WHO recommendations (see Appendix 2).

7.5 Amounts of DNA to be injected:

	Number of Patients	mcg of DNA
A-1	3	10mcg day 1 only
A-2	3	50mcg day 1 only
A-3	3	250 mcg day 1 only

Schedule B
(Will be initiated after safety of schedule A at 50mcg has been established)

	Number of Patients	mcg of DNA
B-1	3	10mcg day 1 and 15
B-2	3	10mcg day 1, 15 and 30

7.6 Criteria for re-treatment: Patients with a partial response at 4 weeks after the last injection of their initial course may receive an additional course of treatment identical to the first treatment, i.e., on Schedule A, at 4 weeks a responding patient may receive a second injection at the same dose initially given; on Schedule B, 4 weeks after two injections (B-1) and 4 weeks after three injections (B-2), responding patients may receive a second course identical to their first course.

7.7 The primary objectives of this investigation are to determine the feasibility and safety of intralesional injections of DNA/lipids into patients with renal cell carcinoma. Patients will be entered in groups of 3 at each dose level and observed for at least 4 weeks before entry of additional patients.

Number of patients with grade 3 toxicity (World Health Organization [WHO])	Instructions for entry of additional patients
0	Escalate to next volume level
1	Enter 3 more patients at same volume level
2 or 3	Discontinue further patient entry at that level and enter 3 more patients at the previous level

8.0 Treatment Modification Based on Toxicity

Toxicity	Dose Reduction
(For liver metastases only) SGOT, SGPT or alk phos >3x baseline	50%
Direct bili 0.2mg/dl above UNL	50%

Other Rx complications or medical conditions which substantially risk to patient	No further Rx
Symptomatic tumor progression requiring other Rx e.g. RT or chemo	No further Rx

9.0 Ancillary Treatment

Symptomatic care may be given as required with medications such as antiemetics and analgesics. However, administration of corticosteroids will require that the patient be removed from study.

10.0 Toxicity Monitoring and Adverse Reaction Reporting

Patients will be monitored and questioned at every visit (see Schedule of Events)regarding the occurrence and nature of any adverse experiences. An event is any change in the physiological or psychological state other than the primary condition that qualifies the patient for this study.

The Investigator must report to Mr. Steven Kradjian (ph 619-453-9777, available 24 hours.) upon occurrence of any life-threatening events (Grade IV) that may be attributable to administration of the study drug, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 3 working days to:

Steven A. Kradjian, Director
Regulatory Affairs, Vical Inc.
9373 Towne Centre Dr., Suite 100
San Diego, CA 92121

Patients will be taken off study immediately if:
Unacceptable toxicity (Grade III or IV) develops and is not easily corrected (refer to WHO toxicity sheet).
Development of Progressive Disease (see definition) requiring the institution of alternative treatments such as radiation, surgery or other drug therapy. If the Investigator believes that the patient's best interest equires a change of therapy.
At the patient's request.

10.1 Toxicities to be graded at each evaluation and pre-treatment symptoms/conditions to be evaluated at baseline per WHO Criteria:

Toxicity/symptoms	Baseline	Each Evaluation
Fatigue	X	X
Weight loss/anorexia	X	X
Nausea	X	X
Vomiting	X	X
Hemorrhage		X
Infection		X

Liver (SGOT, alk phos, and total bilirubin)	X	X

11.0 Treatment Evaluation

11.1 Patients will be evaluated by in the Hematology/Oncology clinic at the University of Chciago at least every 4 weeks while receiving treatment, every 8 weeks until there is evidence of tumor progression, and then 8-12 week intervals thereafter as long as the patient is able to return to the University of Chicago.

11.2 Evaluation of tumor regression is a secondary endpoint in this clinical trial. The following criteria will apply to measurable indicator lesions:

11.3 Minimum size requirements for measurable indicator lesions:

11.3.1 Tumor masses with clearly defined bidimensional measurements. The minimum size of the indicator lesion depends on the method of measurement as follows:

Method of Measurement	Minimum Size of Largest Tumor Diameter
Physical examination or chest x-ray (discrete lesion that can be measured with calipers)	1.0 cm
CT Scan*	3.0 cm

* Note: Special attention should be paid to identify the specific lesion being measured, and to define an anatomic landmark to identify the level of the cross-sectional image to facilitate serial measurements. Lesions 2.0-3.0 cm in diameter may be used as indicator lesions if serial images are obtained at 0.5 cm intervals through the tumor.

11.4 Criteria for a partial response (PR).

11.4.1 Bidimensional indicator lesion(s).

50% reduction in the sum of the products of the largest perpendicular diameters of the indicator lesion(s), single or multiple sites, chosen prior to therapy. Response status will be determined separately for the injected tumor nodule and other measurable lesions that are not injected.

11.5 Criteria for complete response (CR).

Total disappearance of all evidence of tumor.

For a patient to qualify for complete response or partial response, none of the factors constituting progression may be present (see below).

11.6 Criteria for progressive disease (PD):

Tumor progression will be declared if one or more of the following criteria are met.

11.6.1 Appearance of new lesion(s).

11.6.2 Increase in tumor size.

11.6.2.1 Patients with measurable indicator lesion(s) who have met the criteria for partial response.

Fifty percent increase in the size of indicator lesion(s) compared to the smallest measurements while on study.

11.6.2.2 Patients with measurable indicator lesion(s) who have met the criteria for complete response. Progression will be declared for reappearance of any tumor.

11.6.2.3 Any new lesions.

11.6.3 Significant clinical deterioration that cannot be attributed to treatment or other medical conditions.

11.6.3.1 Weight loss >5% body weight.

11.6.3.2 Worsening of tumor-related symptoms.

11.6.3.3 ECOG Performance status decline >1 level

11.7 Criteria for stable (SD)
Failure to meet the criteria for complete response, partial response, regression, or progression.

12.0 Descriptive Factors
None

13.0 Treatment/Follow-up Decision at Evaluation of Patient
Patients meeting the criteria for partial response 4 weeks after their single Schedule A injection or after their last Schedule B injection on may be retreated once.

14.0 Pharmacologic/Immunologic Studies

14.1 Immunochemical staining will be done iat a central laboratory facility as specified by VICAL, Inc. Pre-and post-treatment tumor cells are stained with anti-HLA-B7 antibodies, ME-1, BB7.1, and GSP5.3 (G. Nabel, personal communication) to look for expression.

14.2 Presence of DNA from the HLA-B7 gene will be assessed by PCR amplification of cells obtained by biopsy of the treated site on days 8, 15, and 29 after injection of DNA/lipid complex. Genomic DNA is isolated by standard methods and a portion of the HLA-B7 gene is amplified and sequenced. Several primer sequences may be used (G. Nabel, personal communication). These studies will be carried out in a central laboratory .

14.3 Development of circulating antibodies to HLA-B7 will be evaluated. Autologous peripheral blood B lymphocytes will be EBV immortalized and subjected to *in vitro* gene transfer with the DNA/lipid complex. these autologous cells expressing the HLA-B7 gene will be used to assess the specificity of antibody response to the *in vivo* transfer of the gene. Evidence of cytolytic T-cells will be assessed if a sufficient amount of material is available for successful expansion of infiltrating T-cell population from cells in biopsy of the metastasis. These studies will be carried out in a central laboratory.

15.0 Statistical Consideration and Methodology
Descriptive statistics only will be performed due to the small number of patients.

16.0 Pathology Considerations

16.1 Patients entering this trial will already have had histologically-documented metastatic renal cell carcinoma. A biopsy is carried out as part of this

protocol including the initial one to demonstrate unequivocally by histologic examination of a frozen section that the metastasis selected for injection of the DNA/lipid complex is, in fact, metastatic cancer. Biopsy samples are never tobe placed in fixative.

16.2 For biopsies in which a core of tumor has been obtained, the sample will be subdivided and at least half frozen in liquid nitrogen. The remainder may be subjected to tissue disruption in an attempt to obtain and expand T-cells present in the tissue.

17.0 Records and Data Collection Procedures

17.1 University of Chicago patient records.

17.1.1 The University of Chicago medical records will be utilized for patients. Data will be entered into a data management file within 3 weeks after each evaluation of the patient. After the patient goes off treatment, follow-up information will be collected and entered into the data management file every 3 months.

17.1.2 Pathologic diagnosis made by frozen section is reported in a conventional way with a record being placed in the patient's permanent medical record and data management file.

17.1.3 Data from the central laboratory will be entered into the data management file. Summary reports of samples received will be sent to the principal investigator monthly.

17.2 Vical records.

Case report forms, regulatory documents and source documents will collected and reviewed in accordance with FDA Good Clinical Practices.

Appendix 1

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THE UNIVERSITY OF CHICAGO
DIVISION OF BIOLOGICAL SCIENCES

Informed Consent

Consent By Subject For Participation in Research Protocol

Protocol Number: _____ Patient Name: _____

Title of Protocol: Phase I Study of Immunotherapy for Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions

Doctor(s) Directing Research: Nicholas J. Vogelzang, M.D. (312-702-6743)
Timothy M. Lestingi, M.D. (312-753-3636)
Gary Sudakoff, M.D. (312-702-2562)

You are being asked to participate in a research study. The doctors at the University of Chicago Hospitals and the Division of The Biological Sciences study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent. This consent form gives detailed information about the research study which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

I. NATURE AND DURATION OF PROCEDURE(S):

As you are aware, your kidney cancer cannot be cured by medicine, surgery, or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new treatment which will attempt to induce tumor regression in patients with widespread kidney cancer. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein -- known as HLA-B7 -- causes the cells which contain it to be recognized as "a foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this treatment will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of therapy which stimulates your lymphocytes is called immunotherapy.

Before receiving this treatment, you will receive many tests to

see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests; 2) x-rays of the brain, chest, and abdomen; 3) a blood test for hepatitis; and 4) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV or hepatitis virus, you may not participate in this study. These initial blood tests require that about 45 ml (3 tablespoons) of blood be taken.

If you qualify for this study, we will inject the experimental mixture containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to four times. The time of treatment is usually ~30 minutes. The treatment may be repeated every two weeks for a total of three. Blood samples (about 45 ml or 3 tablespoons) will be obtained daily at first, then weekly for the first month, and biweekly for the next month. A CT scan will be performed before initiation of each treatment and at the end of the two month study period. Your blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of two weeks up to four times.

You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors.

Follow-Up

After you receive the treatment, you will be discharged from the hospital after a one night stay if you have no other significant medical problems. You will be required to return to the University of Chicago Medical Center for follow-up studies described above for at least eight weeks after the trial has begun. It is possible that we may ask you to return after this time if additional tests will be needed. We also will maintain life-long telephone contact with you or your doctor if you are not receiving care at the University of Chicago so that we may check on your progress. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your

condition to deteriorate could be encountered. You will be closely monitored for such side effects.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks of side effects encountered. Your physicians feel that the risks of your disease are much greater than the risks of treatment as outlined above. Furthermore, you physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy, or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

II. POTENTIAL RISKS AND BENEFITS:

Risks and Side Effects

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from the needle injections or tumor biopsies. You may have mild discomfort and bleeding from the tumor biopsy. You will be given a local anesthetic to minimize the discomfort. Second, even though the DNA inserted into your tumor is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could be come abnormal after long periods of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treatment any potential bacterial infections. Finally, there could be bruising at the site where you will be getting your blood drawn as well as possible inflammation or infection of the vein. Of course, care will be taken to avoid these complications.

We emphasize that this procedure, called direct gene transfer, has been used in only a few human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur. This new form of therapy may attract considerable attention from the media and scientific community; however, your identity will be protected from the media and from scientific publication.

No data exists regarding the effects of this therapy on human reproductive function. Therefore, you should use adequate contraception to avoid the pregnancy of you or your spouse during the course of this treatment.

III. ALTERNATIVE THERAPIES:

There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms.

These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

IV. COSTS:

You will not be paid to take part in this study.

Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinic visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The costs of tests and treatments unrelated to this study will be handled as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.

CONSENT FOR AUTOPSY

To fully evaluate the effects and safety of gene therapy, it will be necessary to obtain as much information as possible. In the unlikely event or occurrence of your death, either during this protocol or after its completion, evaluation of your organs will be a very valuable method to see the full effects of gene therapy. Therefore, if you participate in this study, we would like you to consider an autopsy in the event of your death. However, consent to an autopsy is not required to enroll on this trial.

AGREEMENT TO CONSENT

The research project and treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my acces to health care services normally available at the University of Chicago Hospitals. Confidentiality of medical records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by applicable government agencies, including The Federal Food

and Drug Administration, National Cancer Institute and the sponsor of the study, VICAL.

I understand that in the event of physical injury resulting from this research, The University of Chicago Hospitals will provide me with free emergency care, if such care is necessary. I also understand that if I wish, The University of Chicago Hospitals will provide non-emergency care, but the Medical Center assumes no responsibility to pay for such care or to provide me with financial compensation.

I, the undersigned, hereby consent to participate as a subject in the above described research project conducted at The University of Chicago Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have questions concerning my rights in connection with the research, I can contact the Institutional Review Board, at 312-702-1472.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor: _____ _____
Signature of Subject

Witness: _____ _____
Signature of Parent or Guardian
(if patient is a minor)

Date: _____ Time: _____ am/pm

I wish to undergo autopsy in the event of my death: _____ Yes _____ No

(If you answered "Yes" please also inform your next of kin.)

Revision date: 12/21/93

GRANT NUMBER: _____

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators. **CVs**
Copy this page for each person.

NAME	POSITION TITLE
Nicholas J. Vogelzang, M.D.	Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Trinity Christian College	B.A.	1971	Medicine
University of Illinois at Chicago	M.D.	1974	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1974-78 Intern, Resident, Chief Resident - Internal Medicine, Rush Presbyterian St. Luke's Medical Center, Chicago, Illinois

1978-82 Medical Oncology Fellow, Instructor, Assistant Professor - University of Minnesota

1982-87 Assistant Professor of Medicine and Surgery (Urology) - University of Chicago

1987-present Professor of Medicine and Surgery (Urology) - University of Chicago

Honors/National Committees:

American Society of Clinical Oncology (ASCO)
- Program Committee, 1985-86
- Public Relations Committee, 1987-89
- Chairman, Patient Advocacy Committee, 1989-9
- Board of Directors, 1993-96

American Cancer Society, National/State (ACS)
- Illinois Board of Directors, 1987-94
- Second Vice President, 1990-92
- Executive Committee, 1990-94
- Organizing Committee for National Conference on New Oncologic Agents, 1991
- First Vice President, 1992-94
- Scientific Advisory Committee on Clinical Investigation IV, 1992-present

Cancer and Leukemia Group B (CALGB)
- Chemotherapy Committee, 1985-86
- Data Audit Committee, 1985-90
- Respiratory Committee, 1986-present
- Vice Chairman, 1988-90
- GU Working Committee Chair, 1988-93
- Principal Investigator Chicago, 1988-present
- Nominating Committee for New Chairman, 1988-89
- Executive Committee, 1990-93

Publications: *(Total of 20 of 111 peer-reviewed papers, 17 reviews, 27 book chapters, 166 abstracts)*

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HUMAN GENE TRANSFER PROTOCOL

9403-072 Hersh, Evan; Arizona Cancer Center, Tuscon, Arizona; and Akporiaye; Harris; Stopeck; Unger; and Warneke; the University of Arizona, Tuscon, Arizona; *Phase I Study of Immunotherapy of Malignant Melanoma by Direct Gene Transfer.*

Date of RAC Approval: 3/4/1994
Date of NIH Approval: 4/19/1994

Scientific Abstract

The goal of immunotherapy is to stimulate the immune system by modification of tumor cells or expansion of lymphocytes which respond specifically to tumor antigens. In this study, we will apply techniques of direct gene transfer to enhance immune response against tumors *in vivo*. Patients with advanced cancer who have failed all effective therapy will be treated by injection of a DNA/lipid complex directly within the tumor. DNA will be used which encodes a heterodimeric cell surface protein recognized in the transplantation response. These genes include the HLA-B7 histocompatibility antigen and β-2 microglobulin gene in a non-viral plasmid eukaryotic expression vector. For this vector, a safe and effective dose to introduce this recombinant gene in HLA-B7 negative patients will be established. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. We will also determine whether this treatment facilitates tumor regression. This study employs the same study drug as Dr. Gary Nabel of the University of Michigan, previously proposed gene therapy protocol, but expands the study to two other clinical sites, the Mayo Clinic and the Arizona Cancer Center. These studies will facilitate the development of other approaches, using different recombinant genes or in combination with cytokines or adoptive T-cell therapy, to augment tumor immunity, and allow for greater potential efficacy. This method will also establish the safety on this non-viral approach to gene therapy, which could potentially be extended to treat a variety of other human diseases.

Non-technical Abstract

Many types of cancer cannot be cured by traditional medical treatments, including drugs, surgery, or radiation. In this study, an experimental treatment will be offered that may help to fight this disease. We will attempt to induce tumor regression by the introduction of genetic material that directs the synthesis of proteins which stimulate the immune system. The genetic material, DNA, will be introduced directly into the tumor by mixing it with fatty substances, or lipids, and this mixture will be injected into the tumor. The DNA will be taken into cells and cause them to produce proteins that stimulates tissue rejection. These proteins, called histocompatibility proteins, cause cells which contain it to be recognized as foreign by the immune system. The goal of the treatment is to stimulate the immune system to attack and kill the tumor. In this study, we will attempt to determine the optimal safe and effective dose to administer the DNA/lipid complex. Increasing amounts of this complex will be used in different patient populations. If no side effects are observed, repeated treatments will be instituted. The expression and nature of the immune response will also be characterized. This treatment may provide a therapeutic effect in cancer and could be applied to the treatment of other diseases.

PROTOCOL

PHASE I STUDY OF IMMUNOTHERAPY OF MALIGNANT MELANOMA BY DIRECT GENE TRANSFER

PRINCIPAL INVESTIGATOR:

Evan M. Hersh, MD
Hematology/Oncology
Arizona Cancer Center
Tucson, AZ 85724

CO-PRINCIPAL INVESTIGATORS:

Emmanuel Akporiaye, Ph.D.
David Harris, Ph.D.
Alison T. Stopeck, MD
Evan C. Unger, MD
James A. Warneke, MD

Sponsor:

Vical Inc.
Steven A. Kradjian, Director
Regulatory Affairs
9373 Towne Centre Drive
Suite 100
San Diego, CA 92121

Date:

October 5, 1993

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PHASE I STUDY OF IMMUNOTHERAPY OF MALIGNANT MELANOMA BY DIRECT GENE TRANSFER

1.0 Background and Rationale

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells, are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5-10% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor cells with high efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to modulation of immune function, because the immune system can be induced to recognize tumor associated and tumor specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have given about a 10-20% response rate. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive transfer of cytotoxic T cells, which have shown promise in animal models (1-6) and in man (7-10). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy.

Nabel and colleagues at the University of Michigan are investigating a novel molecular genetic intervention for human malignancy that enhances the immune response to tumors by in vivo gene transfer. This immunotherapeutic approach based on animal

model work (11, 12) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7, introduced into human tumors in vivo by DNA/lipid complex transfection. The direct intratumoral injection approach is used. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells. The introduction of an allogeneic MHC gene directly into tumors in vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens.

In a preliminary trial in humans with malignant melanoma Nabel treated 5 patients with malignant melanoma. Three patients received 3 treatments, totaling 0.87 μg of DNA intratumorally, and 3 patients (2 additional patients plus one of the original 3 patients) received cumulative dose of 2.58 μg of DNA via three treatments. No toxicity resulted from this form of treatment and there was no formation of anti DNA antibody or autoantibody. There was no plasmid DNA detectable in the blood by PCR following gene transfer (tested on days 3-7 post transfection at ~2 pg/ml sensitivity).

Evidence of gene transfer was found on biopsy of the injected tumor. The biopsy samples were analyzed for plasmid DNA, mRNA coding for HLA-B7 and the expression of HLA-B7 protein. In 4 of the 5 patients, plasmid DNA and HLA-B7 mRNA were detected within the treated nodules by PCR. HLA-B7 expression was confirmed in all treated nodules by immunohistochemical staining with a monoclonal antibody to the gene product. Two patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells. One of the 5 patients had a partial remission which involved cutaneous and visceral disease. (Nabel, et al., PNAS, in press).

These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

Several improvements that may increase the convenience, safety and efficacy of the procedure have been introduced since the original Nabel studies including:

- o an improved cationic lipid formulation, DMRIE/DOPE*;
- o DNA plasmid construction to optimize expression

The efficacy of transfection was improved for the following reasons. Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner (Vical)

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* DMRIE: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium DOPE: dioleoyl phosphatidylethanolamine bromide.

in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency in vitro compared to the formulation previously used by Nabel. More importantly, this formulation does not aggregate at high concentrations. This characteristic thus allows higher absolute concentrations of DNA and lipid complex to be introduced into experimental animals without toxicity. Because of these properties, it now becomes possible to introduce 100-1000 times more DNA which could allow the study of an expanded dose response gene expression in vivo.

The vector improvements are divided into two categories for this proposal. In the first case expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the β-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface. This is important because some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. It has been found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. These modifications have been incorporated in the study drug to be used in this submission. The study drug is identical to the study drug fully characterized in Dr. Nabel's RAC submission of June 7, 1993, which was unanimously allowed. This study will investigate the administration of the study drug in malignant melanoma.

1.2 Background

1.2.1 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery in vivo provided a model system for the introduction of recombinant gene-specific sites in vivo. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (13,14). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA lipid complex (12). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a

granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site in vivo. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene in vivo (12).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product in vivo. In addition, this approach provided a general method for the induction of a specific site in vivo. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy was developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (11). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.2.2 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (15,16) and lymphokine-activated killer (LAK) cells (17). Cytolytic T cells which infiltrate tumors have been isolated and characterized (18). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (3,19). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (20,21), or by activation induced by substances such as BCG (22).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-α (TNF-α) (23) and lymphotoxin (24) can result in neoplastic cell death. Interferon-γ (IFN-γ) markedly increases class I MHC cell surface expression (25,26) and synergizes with TNF-α in producing this effect (27). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (28), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor

cells but not normal cells (17,29,30). The LAK cells lyse tumor cells without preimmunization or MHC restriction (31). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (32).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanism to escape host defenses, perhaps through evolution of successively less immunogenic clones (33). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (34-38). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (39-43). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (40,41). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (44).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (45), or IL-2 with LAK cells (46,47). Clinical trials using tumor infiltrating lymphocytes are also in progress (48). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (49). Expression of Il-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (50). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

Nabel and co-workers have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (51-55), or expression of allogeneic antigens introduced by somatic cell hybridization (56,57). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection in vitro. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene ($H\text{-}2L^d$) into an $H\text{-}2^b$ tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (58). In another instance,

transfection of H-2^b melanoma cells with the H-$2D^d$ gene did not lead to rejection (59), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (60). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (61). Several subclones which were selected in vitro and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggests that clone-to-clone variation in in vivo growth and tumorigenic capacity may result in other modifications of cells caused by transfection or the subcloning procedure, which affects their tumorgenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly in vivo.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in our animal model studies designed to enhance the immunogenicity of tumors in vivo. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by in vivo infection using retroviral vectors or by DNA lipid complex mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection in vivo.

2.0 OBJECTIVES

2.1 To determine safety and toxicity of direct intralesional injection of increasing amounts of a DNA/lipid mixture: VCL 1005 (HLA-B7/DMRIE/DOPE) into patients with metastatic malignant melanoma. Escalating treatment regimens will be used and tumor growth evaluated.

2.2 To measure the cytotoxic T cell activity directed towards antigens on tumor cells other than HLA-B7.

2.3 To measure humoral and cellular immune responses to HLA-B7.

2.4 To confirm expression in vivo of the HLA-B7 gene in the tumor cells.

2.5 To characterize the clinical response to escalating doses of the study drug by assessing the size of the injected tumor and of all other tumor masses that may be present.

3.0 STUDY DESIGN

This is a Phase I open-label multi-center study in which fifteen patients per center will be enrolled for direct injections with lipid-formulated HLA-B7 plasmids. Each center will enter patients with one tumor type. The Arizona Cancer Center will enroll patients with melanoma. There will be two study arms, single injections (arm 1) and multiple injections (arm 2). Eligible patients will have one solid tumor nodule injected with the study drug (in the single-dose phase of the study), whereas patients enrolled in the repeated-dose phase of the study will have either two or three nodules injected.

Increasing doses of the study drug will be administered in the single-dose phase of the study. Three dosage groups with three patients each will be studied sequentially with at least one month of observation post-injection on the first patient in the dosage group prior to advancing to the next higher dosage.

Initially, Arm 1 patients will receive a single injection of 10 µg of the study drug. Following completion of the first dosage group of three patients, a second single dosage group of three patients will be treated at the second level and the repeated dose arm of the study will be initiated. Group II will receive a five-fold higher concentration of study drug (50 µg). Following completion of the second single dosage group of three patients, a third group of three patients will be treated. Group III will receive a 5-fold higher dose (250 µg) than Group II patients.

The dose will be raised to the next higher level if none of the three patients exhibit any toxicities of grade III or higher. If a patient displays toxicities of grade III or higher, then that dose will be repeated on three additional patients. This arm of the study is summarized in the following table:

ARM 1

Dosage Group	No. of patients per group	Dose per treatment	Total no. of treatments per patient
I	3	10 µg	1
II	3	50 µg	1
III	3	250 µg	1

Patients in arm 2 will receive repeated injections of 10 µg of the study drug. The dose schedule given below will be carried out and second and third doses will be administered to each subject in whom no grade III or greater toxicities are observed. Second and third doses will be administered in different tumors than the first dose if such are available. If not, they will be administered in the same tumor nodule.

ARM 2

Dosage Group	No. of patients per group	Dose per treatment	Total no. of treatments per patient	Days between treatments
I	3	10 µg	2	15
II	3	10 µg	3	15,14

Arm 1 patients will be followed for 8 weeks with a tumor staging at that time. Arm 2 patients will be followed for 10 weeks with a tumor staging at that time. See Appendix 1 for the schedule of events.

4.0 PATIENT ELIGIBILITY

Patients will be carefully selected based on their past medical history and present status. The Investigator and the patient will make a joint decision regarding the appropriate treatment with conventional therapy. Patients will be selected who have failed on conventional therapy or for whom conventional therapy is not indicated.

4.1 Inclusion Criteria

4.1.1 Diagnosis :

Histologically confirmed metastasis of malignant melanoma.

Histologic confirmation may be by needle biopsy at the time of intratumoral injection.

4.1.2 Patients must have at least one metastatic lesion measurable in two dimensions and at least 1 cm in longest diameter.

4.1.3 Patients must have had either prior standard therapies for their disease and have become unresponsive to them or have made the decision that other therapy would not be of any benefit.

4.1.4 Patients must be adults 18 years of age or older.

4.1.5 Patients must be HLA-B7 negative.

4.1.6 Patients must have adequate bone marrow reserve: WBC >3000/mm^3, platelets >100,000/mm^3, hemoglobin >9.0 gm% and adequate renal and liver function, creatinine $\leq$ 2.0 and bilirubin < 2.0 mg/DL.

4.1.7 Patients must have a baseline Karnofsky Performance Status (KPS) score of at least 70. (Appendix 2)

4.1.8 Estimated life expectancy of at least 12 weeks.

4.1.9 Patients must be anti-HIV antibody negative.

4.1.10 Investigator must have obtained signed informed consent from patient.

4.1.11 If patient is female, she must either not be of child-bearing potential by reason of surgery, radiation or menopause or, if of child-bearing potential, using an approved method of contraception.

4.1.12 Patients must be immunocompetent by having at least 2/7 positive skin tests on the Merieux Multitest and by having a PHA lymphocyte response in the normal range.

4.2 Exclusion Criteria

4.2.1 Patients with an active autoimmune disease.

4.2.2 Patients with active hepatitis B (chronic or acute) or HB_S Ag positive, and patients who are HIV sero-positive.

4.2.3 Patients with any active infection requiring parenteral antibiotics.

4.2.4 Radiation, chemotherapy, steroidal therapy within the past three weeks. Surgery within the past 2 weeks.

4.2.5 Patients receiving concurrent anticancer drug therapy, any immunosuppressive drugs and any other experimental therapy.

4.2.5 Immunoincompetence as defined above in 4.1.12.

4.2.7 Patients with diabetes mellitus who are not controlled by medical treatment.

4.2.8 Patients with significant psychiatric disorders that would make compliance to the Protocol difficult or would compromise ability to give informed consent.

5.0 STUDY DRUG

The study drug (VCL-1005) will be supplied as three sterile vials containing (i) HLA-B7 plasmid DNA, (ii) DMRIE/DOPE lipid mixture and (iii) lactated Ringer's. All components will be stable for at least eight weeks under recommended storage

conditions (DNA -20°C, DMRIE/DOPE 4°C). The materials will be supplied by Vical Inc.

5.1 VCL 1005 (HLA-B7 Plasmid DNA)/DMRIE/DOPE:

This study drug is composed of plasmid DNA coding for the complete human MHC HLA-B7 formulated with the cationic lipid mixture DMRIE/DOPE (lipid complex formulation). The DNA concentration is 1.0 mg/ml (see Investigator's brochure for complete details of product characteristics and preparation).

5.2 Lactated Ringer's :

Ready available at the site.

6.0 STUDY DRUG ADMINISTRATION AND TOXICITIES TO BE MONITORED

DNA/lipid complexes are prepared immediately prior to administration. DNA is supplies in 1.0 mg/ml concentration in 400 µl lactated Ringer's solution. Lipid (DMRIE/DOPE) is supplies as a dried film. Each vial contains 77 µg DMRIE and 90 µg DOPE. Each vial is reconstituted with 400 µl lactated Ringer's solutions by vortexing until homogeneous. The contents of the lipid vial is transferred into the DNA vial and mixed well by repeated inversion. The final concentration of the HLA-B7 plasmid DNA is 500 µg/ml. The amount injected into each tumor will be 0.5 ml, for the highest dose of 250 µg. Lower doses, 10 and 50 µg, will be prepared in a similar fashion or formulated as dilutions with lactated Ringer's.

Tumor lesions will be selected for treatment if they are accessible to intratumor administration by direct needle injection. These metastatic lesions will be located at any accessible site such as skin, nodes, lung, liver, spleen. If necessary, the study drug will be injected with the aid of sonographic or CAT scan visualization of the metastasis. Prior to injection following placement of the needle, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm Hg, the injection will be terminated immediately and the patient will be closely monitored or treated appropriately until blood pressure is normalized.

Within 1 hour after the injection, a blood sample will be obtained to check serum enzymes, blood chemistries and cell counts, and to analyze by PCR for the presence of HLA-B7 plasmid DNA in the peripheral blood. Every patient will be checked at 48 hours and another blood collection will be drawn as described in Section 7 below. If any abnormalities appear, the patient will be closely observed. All toxicities will be

graded according to the World Health Organization (WHO) recommendations (see Appendix 3).

7.0 SAFETY AND EFFICACY

No more than two weeks prior to entry into the study, and several times during the study according to the Schedule of Events chart (Appendix 1), all patients will have the following studies performed to assess their disease status:

7.1 Physical exam and medical history including height, weight, performance status and tumor measurements (tumor staging and by physical exam (when accessible).

7.2 Chest X-ray.

7.3 EKG.

7.4 CBC, platelet count, differential, PT, PTT.

7.5 Blood chemistries: creatinine, BUN, bilirubin, alkaline phosphatase, LDH, SGOT, SGPT, phosphorus, uric acid, calcium, total protein, albumin, glucose.

7.6 Merieux Multlitest skin tests

7.7 Lymphocyte Proliferative Response to PHA.

7.8 Pregnancy test for women.

7.9 HIV antibody test.

7.10 Hepatitis screen.

7.11 Tumor biopsies.

7.12 CT/MRI/Ultrasound (if relevant) for baseline and to evaluate status of disease.

7.13 Spectial Tests: Blood will be drawn for serum to be analyzed by PCR for the presence of the plasmid and antibody to HLA-B7. PBLs will be isolated and analyzed for their ability to lyse HLA-B7 modified autologous EBV-transformed lines.

7.14 Blood draws during this study will be limited to a maximum of 50 ml each.

8.0 ADVERSE EVENTS AND REMOVAL FROM STUDY

8.1 Patients will be monitored and questioned at every visit (see Schedule of Events) regarding the occurrence and nature of any adverse experiences. An event is any change in the physiological or psychological state other than the primary condition that qualifies the patient for this study.

8.2 The Investigator must report to Mr. Steven Kradjian (ph 619-453-9900, available 24 hours.) upon occurrence of any life-threatening events (Grade IV) that may be attributable to administration of the study drug, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 3 working days to:

Steven A. Kradjian
Vical Inc.
9373 Towne Centre Drive, Suite 100
San Diego, CA 92121

8.3 Patients will be taken off study immediately if:

8.3.1 Unacceptable toxicity (Grade III or IV) develops and is not easily corrected (refer to WHO toxicity sheet).

8.3.2 Development of Progressive Disease (see definition 9.2.4) requiring the institution of alternative treatments such as radiation, surgery or other drug therapy.

8.3.3 If the Investigator believes that the patient's best interest requires a change of therapy.

8.3.4 At the patient's or guardian's request.

9.0 EFFICACY CRITERIA

9.1 Pharmacologic/Immunologic Studies

As part of the measurement of the efficacy in this study, successful gene transfer and expression will be evaluated by molecular genetic and immunological analyses. Several independent techniques will be used to evaluate the presence and expression of the recombinant gene in vivo.

9.1.1 Monoclonal antibodies to HLA-B7 and immunochemistry techniques will be used to detect the presence of the recombinant gene product in biopsied samples. Fluorescence staining of freshly dispersed cells will also be evaluated.

9.1.2 Successful gene transfer will be assessed by the presence of DNA from the HLA-B7 gene as detected by PCR amplification of DNA from cells obtained by biopsy of the treated sites on various days after the injection of the study drug (see Schema). Genomic DNA will be isolated by standard methods and a portion of the HLA-B7 gene will be amplified and sequenced.

9.1.3 Circulating antibodies to HLA-B7 will be measured by immunoassay. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7-positive and-negative cell lines.

9.1.4 Autologous peripheral blood lymphocytes will be immortalized by EBV transformation and subjected to in vitro gene transfer with the DNA/liposomal complex. These autologous cells expressing the HLA-B7 gene will be used to assess the specificity of patients' immune responses to the HLA-B7 antigen. Direct gene transfer and expression of the HLA-B7 gene is expected to sensitize the patient to HLA-B7 and lead to the generation of an immune response to this antigen. Limiting dilution analysis (LDA) will be done to evaluate alterations in the frequency in HLA-B7-specific helper and cytolytic T cells in the peripheral blood following direct gene transfer.

9.1.5 Peripheral blood lymphocytes will be isolated and cryo-preserved prior to, and at 2 to 4-week intervals following, the initial treatment. At the completion of treatment, PBL samples from each time point will be simultaneously evaluated for responsiveness to HLA-B7 by culturing PBLs, under LDA conditions, with autologous EBV-transformed cells transfected with the HLA-B7 gene. Antigen specific elaboration of IL-2 or generation of CTLs to HLA-B7-positive target cells will be the indices evaluated in these studies. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7-positive and -negative cell lines.

9.1.6 In selected instances, lymphocytes will be isolated directly from the tumor, expanded in tissue culture, and analyzed for cytolytic activity. Tumor biopsies at 7 and 14 days after treatment will be analyzed by immunochemistry. An attempt will be made to isolate and expand draining lymph node T cells or TIL cells to test their cytolytic activity.

9.1.7 When possible, autologous cell lines will be derived from a pretreatment tumor specimen and used in a ^{51}Cr-release assay with peripheral blood lymphocytes.

9.1.8 Finally, every attempt will be made to excise tumor tissue prior to treatment for diagnosis, immunochemistry, cryo-preservation and to evaluate delayed-type hypersensitivity reactions to the tumor before and after treatment.

9.2 Clinical Response Criteria

All patients who receive at least one dose of study drug will be analyzed for efficacy and safety. Standard oncologic criteria will be applied to determine whether or not a patient responds to the study drug. All tumor measurements must be recorded in centimeters and should consist of the longest diameter and the perpendicular diameter at the widest portion of the tumor. All the following determinations of tumor response should be made comparing current total tumor size to pre-treatment total tumor size.

9.2.1 Complete Tumor Response

Disappearance of all clinical evidence of active tumor for a minimum of four weeks. The patients must be free of all symptoms of cancer.

9.2.2 Partial Tumor Response

Fifty percent (50%) or greater decrease in the sum of the products of all diameters of measurable lesions. These reductions in tumor size must endure for a minimum of four weeks. No simultaneous increase in the size of any lesion or appearance of new lesions may occur. The appropriate diagnostic tests used to demonstrate the response must be repeated four weeks after initial observation in order to document this duration.

9.2.3 Stable Disease

Less than 50% decrease in the sum of the products of all diameters of measurable lesions, or an increase in the tumor mass less than 25% in the absence of the development of new lesions.

9.2.4 Progressive Disease

Tumor progression will be defined if one or more of the following criteria are met:

- Appearance of any new lesion(s).
- Increase in tumor size of $\geq 25\%$ in the sum of the products of all diameters of measurable lesions.
- Significant clinical deterioration that cannot be attributed to treatment or other medical conditions and is assumed to be related to increased tumor burden.
- Worsening of tumor-related symptoms clinically significant by physician.

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10.0 INFORMED CONSENT AND IRB APPROVAL

The Investigator at each site will be responsible for and shall prepare a written Informed Consent Document. The Protocol and the Informed Consent Document must be provided to his IRB. The IRB must approve the Protocol and Informed Consent Document and agree to monitor the conduct of the study and periodically review its progress at no less than one year intervals. Before the study begins, the Investigator will provide the Sponsor with documented evidence, i.e., a letter from the IRB or a copy of the IRB minutes, of:

- o Protocol and Informed Consent approval.
- o The IRB agreement to monitor the conduct of the study and review it periodically.

10.1 Updates To The Informed Consent Documents

The Sponsor will notify the Investigator should significant new findings develop during the course of the study that may relate to the patient's willingness to continue participation. It is the Investigator's responsibility to:

- o Notify the local IRB.
- o Update the Informed Consent Document.
- o Notify each patient accordingly.
- o Each patient's record must contain a signed copy of the updated Informed Consent Document.
- o Send the Sponsor a copy of each patient's signed updated Informed Consent Document.

11.0 STATISTIC CONSIDERATIONS

Descriptive statistics will be performed due to the small number of patients.

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SUBJECTS' CONSENT FORM Page _1_of_5_

Appr. Date: 11/23/93 TITLE: Phase I Study of Immunotherapy Number:
Rev: 1/13/94 of Malignant Melanoma by SWOG:
Direct Gene Transfer HSC: 93-175

Revised Consent Form

I AM ARE BEING ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT I AM INFORMED OF THE NATURE OF THIS RESEARCH STUDY AND OF HOW I WILL PARTICIPATE1/13/9 IN IT, IF I CONSENT TO DO SO. SIGNING THIS FORM WILL INDICATE THAT I HAVE BEEN SO INFORMED AND THAT I GIVE MY CONSENT. FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT PRIOR TO PARTICIPATION IN THIS RESEARCH STUDY SO THAT I KNOW THE NATURE AND THE RISKS OF MY PARTICIPATION AND CAN DECIDE TO PARTICIPATE OR NOT PARTICIPATE IN A FREE AND INFORMED MANNER.

PURPOSE

I am being invited to voluntarily participate in the above-titled research project. The purpose of this project is to evaluate the toxicity (side effects and safety of gene therapy of malignant melanoma by introducing a manufactured gene substance into a melanoma tumor nodule. It is hoped to induce immunity to the melanoma. However, this is a phase I study which means it is designed to test the safety of 3 different doses of the gene given 1, 2 or 3 times and has no assurance that it will have any benefit, although any effect on tumor size will be observed.

SELECTION CRITERIA

I am being invited to participate because I have metastatic malignant melanoma which means that the tumor (cancer) has spread to lymph nodes (glands) or internal organs such as liver or lung. Furthermore, my tumor is no longer or not amenable to conventional treatment with surgery, radiation or approved drugs.

If I am female I must not be pregnant or nursing. Men and women of child-bearing age and capacity must use effective birth control during study participation (condoms are highly encouraged).

A total of up to 15 patients will be enrolled at the Arizona Cancer Center.

STANDARD TREATMENT(S)

There are no known cures for patients with my disease. Also, before being eligible for this protocol I will have failed on or been unable to take front line chemotherapy which has a 50% response rate. Alternative treatments available to me include the delivery of x-ray treatment to sites of local disease (50%) response rate), medication to control pain (close to 100% response rate) and certain established chemotherapy treatments or experimental drugs are being evaluated at other centers to which I can be referred. These could have response rates in the range of 20% but this is not certain as they are experimental. Experimental treatments are under investigation which attempt to stimulate the immune system to reject the tumor, and I can be referred to physicians who are conducting such trials. In some cases, proteins are injected which can stimulate the immune system. I also have the option to receive no treatment at this time.

PROCEDURE

Within 14 days before I start the test injections, I will have a complete history and physical evaluation, electrocardiogram (EKG), urinalysis, blood work (complete blood count or CBC), differential and platelets, blood chemistries, pregnancy test, hepatitis

test, other HIV test, special other blood tests and my tumor sites will be measured, either by direct measurement or x-ray measurement.

If I agree to participate, I will be asked to agree to the following which is described below.:

If I decide to take part in this study, I will receive my injections as an outpatient. In this study, injections of manufactured genes will be offered that may help to fight this disease in other patients. Because this treatment is experimental, I may not derive any direct benefit from it. The purpose of this study is to determine a safe dose of a new treatment which will attempt to induce tumor shrinkage. Because this is new and experimental, I will be observed to determine the side effects of the therapy. I will also be monitored for the effects of this treatment on the growth of my tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called lipids, and then transport the mixture into my tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which it is hoped, based on animal studies,will stimulate tumor tissue rejection. One protein – known as HLA-B7 – causes the cells which will contain it to be recognized as "foreign enemy" by the immune system. The purpose of the study is to determine whether this treatment will induce the cells of my immune system, known as lymphocytes, to attack and kill the tumor. This type of therapy which stimulates lymphocytes is called immunotherapy.

If I qualify for this study, I will have a solution containing the DNA/lipid complex injected directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and areas within a single nodule will be injected. The time of treatment is approximately 30 minutes. The treatment will be given once or repeated every 2 weeks for a total of three treatments depending on which group of the protocol I am placed. Blood samples (between 1-2 ounces) will be obtained weekly or biweekly. A CT scan will be performed before initiation of treatment and once or twice in the 2-month study period. My blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. My blood will also be tested for evidence of toxicity (side effects) from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 3 times. These tests will be in conjunction with the treatments.

I will be checked regularly for any toxicity (side effects of the drug). Clinic visits will be required approximately every week. In addition, a collection of small quantities of blood (2-6 teaspoons) for laboratory tests will be taken weekly and X-rays every 2 months and at the end of the study. The study may be terminated before that time by patient decision, side effects or if medically indicated. Patient follow-up after study completion will be life-long.

RISKS

All current methods of anticancer treatment (whether standard or experimental) have potential side effects. In studies thus far conducted in a few patients, no side effects have been noted. However, this is so small that the incidence of side effects for this therapy are really not known. Patient follow-up after study completion will be life-long.

I will be kept informed of results from this study while I am receiving the drug, especially regarding any finds which might affect my willingness to participate.

There are potential side effects and risks to this procedure. First, I may experience mild discomfort from needle injections or tumor biopsies. I may have mild discomfort and bleeding from the tumor biopsy. I will be given a local anesthetic to minimize the discomfort. Also, if the injections are into nodules in the liver, bleeding may be serious and require surgery to stop it. If the injections are in the lung area the lung may be punctured requiring hospitalization or a chest tube. There is a risk of death due to bleeding in pneumothorax. Second, even though the DNA inserted into my tumor is considered harmless to me, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and it is not known whether cells could become abnormal after long periods of time. In animal studies, the development of cancer cells has not been observed in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections.

It has been explained to me that this procedure, called direct gene transfer, has been used before in human patients only in one preliminary clinical trial. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur.

I will undergo biopsy of tumor and other tissue, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If this is successful, I will be immunized to the HLA-B7 protein. In the event that I should require an organ transplant, I would not be able to receive an organ from an individual who makes this protein, which occurs in approximately 15% of donors.

BENEFITS

This study should provide the information needed to subsequently conduct a phase II study to determine if this approach will lead to responses. The injections I will receive may induce an immune response to my tumor and a remission (meaning a shrinkage of the cancer for weeks to months).

CONFIDENTIALITY

Medical information about me and my progress in this study will remain confidential. Should the results of this study be reported or published, no individual

patient names will be used. In accord with the requirements of the Food and Drug Administration (FDA), occasionally medical records of patients may be reviewed by medical monitors of sponsoring agencies whose duty it is to oversee this research to verify its accuracy. This may include the sponsoring company (Vical Inc. San Deigo, California), the National Cancer Institute and the FDA.

PARTICIPATION COSTS

The experimental drug HLA-B7 gene in a plasmid liposome (fatty substance) delivery system will be supplied by the study sponsor. I will be charged for the standard tests associated with the delivery of routine medical care for my disease. This includes routine blood tests, X-rays, scans, and physician's charges. The charges associated with the management of the side effects of treatment (medication, etc.) will also be billed to me or a third party carrier. All costs are standard for medical care involving these treatments. The drug and its administration and any additional testing will be covered by the sponsor. I can obtain further information from Evan M. Hersh, M.D. (Principal Investigator) at (602) 626-2250 or page at 694-6000, (pager 1240), Dr. Evan Unger (602) 694-2515 (pager 2042), Dr. James Warneke (602) 626-7754 (pager 2429) or Dr. Alison Stopeck (602) 626-2816 (pager 1231).

LIABILITY

I understand that adverse reactions are possible in any research program despite the use of high standards of care and could occur through no fault of mine or the investigator involved. Reactions which can be foreseen have been described in this consent form. However, unforeseeable harm may also occur and may require care.

I understand that money for research-related side effects or harm or for wages or time lost is not available. Necessary emergency medical care directly related to this treatment will be provided from Evan M. Hersh, M.D. I can obtain further information from Evan M. Hersh, M.D. (Principal Investigator) at (602) 626-2250), Dr. Evan Unger (602) 694-2515 (pager 2042), Dr. James Warneke (602) 626-7754 (pager 2429) or Dr. Alison Stopeck (602) 626-2816 (pager 1231). If I have questions concerning my rights as a research subject, I may call the Human Subjects Committee office at (602) 626-6721.

AUTHORIZATION

BEFORE GIVING MY CONSENT BY SIGNING THIS FORM, THE METHODS, INCONVENIENCES, RISKS AND BENEFITS HAVE BEEN EXPLAINED TO ME AND MY QUESTIONS HAVE BEEN ANSWERED. I UNDERSTAND THAT I MAY ASK QUESTIONS AT ANY TIME AND THAT I AM FREE TO WITHDRAW FROM THE PROJECT AT ANY TIME WITHOUT CAUSING BAD FEELINGS OR AFFECTING MY MEDICAL CARE. MY PARTICIPATION IN THIS PROJECT MAY BE ENDED BY THE INVESTIGATOR OR BY THE SPONSOR FOR REASONS THAT WOULD BE EXPLAINED. NEW INFORMATION DEVELOPED DURING THE COURSE OF THIS STUDY WHICH MAY AFFECT MY WILLINGNESS TO CONTINUE IN THIS RESEARCH PROJECT WILL BE GIVEN TO ME AS IT BECOMES AVAILABLE. I UNDERSTAND THAT THIS CONSENT FORM WILL BE FILED IN AN AREA DESIGNATED BY THE HUMAN SUBJECTS COMMITTEE WITH ACCESS RESTRICTED TO THE PRINCIPAL INVESTIGATORS. EVAN M. HERSH, MD, EVAN UNGER, MD, JAMES WARNEKE,MD OR ALISON STOPECK,MD. AN AUTHORIZED REPRESENTATIVE

OF THE HEMATOLOGY/ONCOLOGY SECTION OF THE CANCER CENTER. I UNDERSTAND THAT I DO NOT GIVE UP ANY OF MY LEGAL RIGHTS BY SIGNING THIS FORM. A COPY OF THIS SIGNED CONSENT FORM WILL BE GIVEN TO ME.

SIGNATURES:

Subject Date Parent/Legal Representative Date

I have carefully explained to the subject the nature of the above project. I hereby certify that to the best of my knowledge the person who is signing this consent form understands clearly the nature, demands, benefits and risks involved in his/her participation. A medical problem or language or educational barrier has not precluded this understanding.

Investigator Date Witness Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Evan M. Hersh, M.D.	Professor of Internal Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
College of the City of New York	B.S.	1956	Medicine
Columbia University, New York	M.D.	1960	Medicine
St. Luke's Hospital, New York	Internship	1961	Medicine
Standford University, Californi	Residency	1962	Medicine
Medicine Branch, NCI, Bethesda, MD	Clin. Assoc	1965	Medicine

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1962 to 1965 Clinical Associate, Medical Branch, NIH, NCI, Bethesda, Maryland

1965 to 1966 Fellow in Medicine & Immunology, Stanford University Medical Center, Palo Alto, CA

1966 to 1972 Assistant Internist & Assistant Professor of Medicine, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Institute, Houston, Texas

1971 to 1981 Chief, Section of Immunology, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Institute, Houston, TX

1972 to 1975 Associate Internist and Associate Professor of Medicine, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital Tumor Institute, Houston, Texas

1975 to 1981 Professor of Medicine and Internist, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Institute, Houston, Texas

1977 to 1981 Deputy Head, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Institute, Houston, Texas

1981 to 1986 Chairman, Professor of Medicine and Internist, Department of Clinical Immunology & Biological Therapy, the University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Institute, Houston, Texas

1986 to Present Chief, Section of Hematology and Oncology and Professor of Medicine, Department of Internal Medicine, Arizona Cancer Center, University of Arizona Health Sciences Center, University of Arizona, Tucson, Arizona

1986 to Present Professor of Microbiology and Immunology, Department of Microbiology and Immunology, University of Arizona, Tucson Arizona

1986 to Present Associate Director for Clinical Research and Director of Immunology, Arizona Cancer Center, Tucson, Arizona

Honors and Awards:

Second Year Honors, City College, New York, 1954

Phi Beta Kappa & Medal for Student of Most Proficiency in Biology, City College, New York, 1956-1960

Salk Scholar, City of New York, 1960

Alpha Omega Alpha, Columbia University, New York, 1981

1000 Contemporary Scientists, Most Cited 1965-1978 Top 300 (Current Contents, 1981), Citation Class (Current Contents), 1983.

Publications:

1. Hersh EM, Miller TP. Malignant lymphomas in patients with human immunodeficiency virus infection, Recent Results in Cancer Research 112:54-62. 1988.

2. Chong ASF, Hersh EM, Grimes WJ. Blocking of lymphokine activated killer (LAK) cell mediated cytotoxicity by cell sized beads bearing tumor cell proteins. J Immunol 141(12):4418-4424, 1988.
3. Chong ASF, Scuderi P, Grimes WJ, Hersh EM. Tumor targets stimulate IL-2 activated killer cells to produce Interferon-g and tumor necrosis factor[1]. J Immunol 142:2133-2139, 1989.
4. Hersh EM, Scuderi P, Grimes WJ, Chong A, Brailey JL, Gschwind CR, Salmon SE. Cytostatic and cytotoxic activity of lymphokine activated killer (LAK) cell supernatants. Cancer Immunol Immunother 30(1):65-70, 1989.
5. Hersh EM, Murray JL, Hong WK, Rosenblum MG, Rueben JM, Wellbaecher R, Sarwal AN, Bradley EC, Arnett FC. Phase I study of cancer therapy with recombinant interleukin-2 administered by intravenous bolus injection. Biotherapy 1:215-226, 1989.
6. Schwartz JE, Scuderi P, Wiggins C, Rudolph A, Hersh EM. A phase I trial of recombinant tumor necrosis factor (rTNF) administered by continuous intravenous infusion in patients with disseminated malignancy. Biotherapy 1:207-214, 1989.
7. Hersh EM, Funk CY, Petersen EA, Mosier DE and the Ditiocarb Study Group. Biological activity of diethyldithlocarbamate (Ditiocarb, Imuthiol[R]) in an animal model of retrovirus-induced immunodeficiency disease and in clinical trials in patients with HIV infection. Develop Biol Standard, 72:355-363, 1990
8. Hersh EM, Brewton G, Abrams D, Bartlett J, Galpin J, Gill P, Gorter R, Gottileb M, Jonikas J, Landesman S, Levine A, Marcel A, Petersen EA, Whiteside M, Zahradnik J, Negron C, Boutitle F, Caraux J, Dupuy JM, Salmi LR. Ditiocarb sodium (diethyldithlocarbamate) therapy in patients with symptomatic HIV infection and AIDS. A randomized double-blind placebo-controlled multicenter study. JAMA 265:1538-1544, 1991.
9. Hersh EM, Funk, CY, Ryschon KL, Petersen EA, Mosier DE. Effective therapy of the LP-BM5 murine retrovirus-induced lymphoproliferative immunodeficiency disease with diethyldithlocarbamate. AIDS Research and Human Retroviruses 7:553-561, 1991.
10. Hersh EM, Metch BS, Muggia FM, Brown TD, Whitehead RP, Budd GT, Rinehart JJ, Crawford ED, Bonnet JD, Behrens BC. Phase II studies of recombinant human tumor necrosis factor alpha (rhuTNFα) in patients with malignant disease. J Immunotherapy 10:426-431,1991
11. Lake D, Kawamura T, Tomiyama T, Masuho Y, Matsumoto Y, Robinson, Jr. WE, HERSH EM:Generation and Characterization of a human monoclonal antibody that neutralizes diverse HIV-1 isolates in vitro. Current Science, AIDS, 6:1724, 1992.
12. Taylor CW, Chase EM, Whitehead RP, Rinehart JJ, Neidhart JA, Gonzalez R, Bunn PA, HERSH EM: A southwest oncology group phase I study of the sequential combination of recombinant interferon-γ and recombinant interleukin-2 in patients with cancer. J Immunotherapy, 11:176-183, 1992.
13. Verdi CJ, Taylor CW, Crogan MK, Dalke P, Meyskens FL, HERSH EM: Phase I study of low-dose cyclosphosphamide and recombinant interleukin-2 for the treatment of advanced cancer. J Immunotherapy, 11:286-291, 1992.
14. Funk CY, Eisman J, Hersh EM: Treatment of the murine, retrovirus-induced lymphoproliferative immunodeficiency disease (LP-BM5) in C57BL/10 mice with the immunomodulator imexon. AIDS Research and Human Retroviruses, 8:633-638, 1992.
15. HERSH EM, Gschwind C, Taylor CW, Dorr RT, Taetle R and Salmon SE: Anti proliferative and anti-tumor activity of the cyanoaziridine compound imexon on tumor cell lines and fresh tumor cells in-vitro. JNCI, 84:1238-1244, 1992.
16. HERSH EM, Grogan TM, Funk CY and Taylor CW: Suppression of human lymphoma development in the SCID mouse by imexon therapy. J Immunotherapy, 13:77-83, 1993.
17. HERSH EM,Funk CY, Petersen EA, Ryschon KL and Mosier, DE: Dose response and timing effects in the therapy of the LP-BM5 murine retrovirus induced lymphoproliferative immunodeficiency disease with diethyldithiocarbamate. Int J Immunopharmac, 15:137-143, 1993..
18. Barbuto JAM, Verastegui EL and HERSH EM: The use of transformed T cell lines for clonal expansion of human B cells from peripheral blood, spleen and tumor infiltrating lymphocytes. Hybridoma. 12:115-125, 1993.
19. Punt CJA, Barbuto JAM, Grimes WJ, and HERSH EM: Anti-tumor antibody production by tumor-infiltrating (TIBL) and peripheral blood (PBBL) human B lymphocytes. Cancer Research (Submitted).
20. Dorr RT, Liddil JD, Dalton WS, Galler L, and HERSH EM: The 2 cyanoaziridine derivative imexon induces protein-associated DNA-single strand breaks in human 8226 myeloma cells. Cancer Research. (Submitted).

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Emmanuel T. Akporiaye	Associate Professor

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Ife, Ile-Ife, Nigeria	B.S.	1973	Biol. Sciences
New Mexico Highlands Univ. Las Vegas, NM	M.S.	1978	Biology
University of New Mexico, Albuquerque, NM	Ph.D.	1983	Path. Micro.

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1973 - 1974 Science Teacher, (Biology and Chemistry). St. Scholastica Girl's High School, Bakana, Port Harcourt, Rivers State, Nigeria. Set up and administered International Diploma Examinations (West African School Certificate Examinations).

1974 - 1975 Science Teacher, Sapele Technical School, Sapele, Nigeria. Was involved in organizing and setting up curriculum for freshmen.

1975 - 1977 Assistant Lecturer in Biology, College of Education, Abraka, Bendel, Nigeria.

1977 - 1978 Teaching Assistant, New Mexico Highlands University, Dept. of Biology, Las Vegas, NM.

1979 - 1983 Research Assistant, University of New Mexico, Department of Biology, Albuquerque, NM, under a grant from the National Science Foundation for the study of mechanism(s) of host-parasite interactions between *Coxiella burnetii* and macrophage-like tumor cell lines.

1983 - 1985 Postdoctoral Research Fellow, Los Alamos National Laboratory, Los Alamos, NM, with Carleton C. Stewart, Ph.D., Experimental pathology Group: "Radiation and the Mononuclear Phagocyte Response to Cancer".

1985 - 1990 Assistant Professor, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

1990 - Present Associate Professor of Microbiology and Immunology, University of Arizona, Tucson, AZ.

HONORS:

Igh School	Ranked first in graduating class
University of Ife:	Received National Award based on merit for four years.
University of	Received best graduate student presentation New Mexico award at New Mexico Branch ASM Meeting (1982).

MEMBERSHIP AND POSITIONS IN PROFESSIONAL SOCIETIES:

The American Association for Cancer Research
Society for Leukocyte Biology
Sigma Xi, The Research Society
New York Academy of Sciences

PUBLICATIONS: (From a total of 21)

1. E.T. Akporiaye, G.C. Saunders, and P.M. Kraemer, A Sponge Model for Studying Tumor Growth: Quantitation of Tumor Cells and Leukocytes in the CHO Tumor. Experientia 43, 589-593 (1987).

2. E.T. Akporiaye, M. Kudalore, A.P. Stevenson, P.M. Kraemer and C.C. Stewart, Isolation and Reactivity of Host Effectors Associated with the Manifestation of Concomitant Tumor Immunity. Cancer Res. 48, 1153-1158 (1988).

E.T. Akporiaye and M. Kudalore. Tumor growth inhibition by T lymphocytes recovered from a site of tumor rejection, Cancer Immunol. Immunother. 29, 199-204 (1989).

4. E.T. Akporiaye, V. Tsosie, D. Stefanovich and O.G. Baca. Coxiella burnetii fails to stimulate superoxide anion production by human neutrophils Acta Virol. 34, 64-70 (1990).

5. E.T. Akporiaye, and C.A. Barbieri, C.C. Stewart and J.G. Bender. Gelatin Sponge model of effector recruitment: Tumoricidal activity of adherent and non-adherent Lymphokine activated killer cells after culture in Interleukin-2. J. Leukocyte Biol. 49, 189-196 (1991)

6. A.L. Heitkemper and E.T. Akporiaye. Chemotactic activity resides primarily in the adherent population of lymphokine activated killer cells. J. Chemotherapy 3 suppl 4, 530-532, (1991)

7. L.L. Steed, E.T. Akporiaye and R.L. Friedman, *Bordetella pertussis* induces respiratory burst activity in human polymorphonuclear leukocytes. Infect. Immun. 60, 2101-2105, (1992).

8. R.L. Friedman, K. Nordensson, L. Wilson, E.T. Akporiaye and D.E. Yocum. Uptake and intracellular survival of Bordetella pertussis in human macrophages. Infect. Immun. 60, 4578-4585 (1992).

E.T. Akporiaye, A. Peterson, P. Pierce, J. Valenzuela, L. Canfield and J. Bender. Effect of Beta-Carotene on cytotoxic activity and Receptor expression of tumor-specific lymphocytes. In Press. Ann. N.Y. Acad Sci. (1993).

10. J. A. Park, R. A. Brown, K. Unverzagt, J.G. Bender, K.W. Girodias and E.T. Akporiaye. Specific recruitment and activation of tumor rejecting lymphocytes during concomitant tumor immunity. Submitted.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

	POSITION TITLE
David T. Harris, Ph.D.	Associate Professor

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Wake Forest University	B.S.	1976	Biology & Mathematics
Wake Forest University	M.S.	1979	Medical Sciences
Wake Forest University	Ph.D.	1982	Microbiology and Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE;

1982-1985 Postdoctoral Fellow, Ludwig Institute for Cancer Research, Lausanne, Switzerland

1985 Staff Immunologist, Environmental Monitoring & Services Inc., Chapel Hill, NC

1985-1989 Research Associate (Research Assistant Professor), Center for Environmental Medicine and Lung Biology, Department of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC

1989-present Associate Professor, Microbiology and Immunology, University of Arizona, Tucson, AZ

1990-present Associate Member, Clinical Scientist, Arizona Arthritis Center

Publications

Evans, D.L., D.T. Harris, H.S. Koren, and L. Friedmann. Identification of a recognition structure on rat natural killer cells. IN: Natural Killer Cells: Biology and clinical application (Proceedings of the 6th International Natural Killer Workshop), R.E. Schmidt, Ed., Karger Press, Basel, Switzerland, p. 5-9, 1990.

Evans, D.L., D.T. Harris, and L. Jaso-Friedmann. Effects of phorbol esters and calcium ionophore on nonspecific cytotoxic cells. Dev. Comp. Immunol 14(2):223, 1990.

Friedmann, L., D.T. Harris, A. St. John, H.S. Koren, and D.L. Evans. Characterization of an evolutionary conserved target cell antigen complex recognized by natural killer cells. IN: Natural Killer Cells: Biology and clinical application (Proceedings of the 6th International Natural Killer Workshop), R.E. Schmidt, Ed., Karger Press, Basel, Switzerland, p. 13-17, 1990.

Harder, S.D., D. House, D.T. Harris, and H.S. Koren. Inhibition of human natural killer cell activity following *in vitro* exposure to ozone. Inhal. Tox. 2:161, 1990.

Harris, D.T., L. Friedmann, R.B. Devlin, K.P. McKinnon, H.S. Koren and D.L. Evans. A putative antigen receptor on human natural killer cells is a signal-transducing molecule. IN: Natural Killer Cells: Biology and clinical application (Proceedings of the 6th International Natural Killer Workshop), R.E. Schmidt, Ed., Karger Press, Basel, Switzerland, p. 10-12, 1990.

Harris, D.T., L. Jaso-Friedmann, R.D. Devlin, K.P. McKinnon, H.S. Koren and D.L. Evans. Identification of an evolutionarily conserved natural killer cell (NK) antigen receptor/target antigen complex. IN: Defense Molecules, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 121, Eds. J. Marchalonis and C. Reinisch, Alan R. Liss, New York. NY, pp. 213-227, 1990.

Harris, D.T., L. Jaso-Friedmann, R.B. Devlin, H.S. Koren and D.L. Evans. Identification of a novel function-associated molecule on human natural killer cells. Proc. Natl. Acad. Sci. USA 88:3006, 1991.

Harris, D.T., L. Friedmann, and D. Evans. 1991. Recognition, receptors and natural killer cells. Preceedings of the 5th International Society of Developmental and Comparative Immunology Congress, Pergamon Press, in press, 1992

Evans, D.L., D.T. Harris, J.H. Leary, L. Friedmann and A. St. John. Identification of a vimentin-like protein on rat NK cells: Evidence for receptor function. Scand. J. Immunol., in press, 1992.

Evans, D.L., D.T. Harris, and L. Jaso-Friedmann. A function -associated molecule on rat natural killer cells identified by anti-idiotypic monoclonal antibodies. Cell. Immunol. 141:293-305, 1992.

Evans, D.L., D.T. Harris, and L. Jaso-Friedmann. Function-associated molecules on nonspecific cytotoxic cells: Role in calcium signalling, redirected lysis and modulation of cytotoxicity. Dev. Comp. Immunol. 16(5):383-394. 1992.

Harris, D.T., L. Jaso-Friedmann, R.B. Devlin, H.S. Koren and D.L.Evans. The Natural Killer Cell-Like Lytic Activity Expressed by Cytolytic T lymphocytes is associated with the expression of a novel function-associated molecule. Scand. J. Immunol. 35:299, 1992.

Harris, D.T. Immunological Analysis of Cord Blood: Implications for Graft Versus Host Disease. Cord Blood Stem Cells: Current Status and Future Prospects, K. Moch, (ed)., p. 36-38, 1992

Harris, D.T.., L. Friedman, and D.L. Evans. Modulation of function-associated molecules on NK cells alters recognition and cytotoxicity. *In* NK Cell Mediated Cytotoxicity: Receptors, Signalling and Mechanisms. Eds. Lotzova, and Herberman, CRC Press, p. 53-65, 1992.

Harris, D.T., M.J. Schumacher, J. LoCacsio, F.J. Bescencon, G. Olson, D. DeLuca, J. Bard and E.A. Boyse. Phenotypic and functional immaturity of human umbilical cord blood T lymphocytes, Proc. Natl. Acad. Sci. USA, in press, 1992.

Harris, D.T., R. Kapur, C. Frye, A. Acevedo, T. Camenisch, L. Jaso-Friedmann and D.L. Evans. A species-conserved NK cell antigen receptor is a novel vimentin-like molecule. Dev. Comp. Immunol. 16(5):395-403, 1992.

Harris, D.T., M.J. Schumacher, J. Locascio, J., F.J. Besencon, G.B. Olson, D. DeLuca, J. Bard, and E.A. Boyse. Phenotypic and function immaturity of cord blood T lymphocytes. Proc. Natl. Acad. Sci. USA, in press, 1992.

Jaso-Friedmann, J.H. Leary, A. St. John, D. T. Harris, H.S. Koren and D.L. Evans. Detection by monoclonal antibodies of function-associated molecules on rat NK cells. II. Biochemical characteristics. Natl. Immun. & Cell Growth Reg. Accepted, 1992.

Jaso-Friedmann, L., A. St. John, D.T. Harris, H.S. Koren and D.L. Evans. Detection of function-associated molecules on rat NK cells and their role in target cell lysis. Cellular Immunology, 141:131-147, 1992.

Olson, G.B., J. Bard, D.T. Harris, E. Spooncer, T.M. Dexter, and E.A. Boyse. Cell Cycle analysis of stem cells: Detection by fluorescent and non-fluorescent dyes and digital image processing. Analytical and Quantitative Cytology and Histology, in press, 1992.

Yeoman, H., R.E. Gress, M. Ogawa, E.A. Boyse, J. Bard, D.T. Harris., and D. DeLuca. Development of human stem cells in murine fetal thymus organ culture. Proc. Natl. Acad. Sci. USA, in press. 1992.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Alison T. Stopeck	Clinical Assistant Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Brown University, Providence, RI	B.A.	1981	Biochemistry
Columbia Univ. College of Physicians & Surgeons, New York, NY	M.D.	1985	Medicine

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Medical Training

1985-1988	Medical intern and resident, Columbia-Presbyterian Medical Center
1988-1989	Clinical Fellow, Hematology-Oncology, New York Hospital-Cornell Medical Center
1990-1992	Instructor in Medicine, Cornell University Medical College
1992-1993	Assistant Professor of Medicine, Cornell University Medical College
1993-Present	Clinical Assistant Professor of Medicine, University of Arizona

Research

1982	Research Assistant, Brown University
1984	Medical Student Research Elective, Columbia Univ. Dept. of Medicine, Division of Nephrology
1987	Senior Resident Research Elective, Columbia Presbyterian Medical Center, Dept. of Medicine, Division of Endocrinology
1989-1993	Research Fellow, Cornell Medical College, Hematology-Oncology, Laboratory of Vascular Biology

Academic Honors and Awards:

Magna Cum Laude
Sigma XI
N.Y. State Physicians' Scholarship
Rudin Scholar - College of Physicians and Surgeons
Alpha Omega Alpha

Memberships

American College of Physicians
American Federation of Clinical Research
American Society of Hematology

Board Certification

1988 Internal Medicine
1990 Hematology
1991 Oncology

Bibliography:

PUBLICATIONS:

GL Dettman,. AT Stopeck, BW Meyer, AH Iosiphidis. In vivo migration of ^{111} in radiolabeled mouse-spleen lymphocytes and tumor cells. Int. J. Nuclear Medicine and Biology. Vol. 10, No. 1 pp 11-16, 1983.

JH Godwin, AT Stopeck, VT Chang, TA Godwin. Mycobacteremia in AIDS: rapid diagnosis based on inclusions in the peripheral blood smear. Amer. J Clin. Path. Vol. 95, pp 369-375, 1991.

HH Kazarian Jr, CE Dowling, RL Hurwitz, M Coleman, AT Stopeck, JG Adams III. Dominant Thalassemia-like phenotypes associated with mutations in exon 3 of the ß-globin gene. Blood. Vol. 79, pp 3014-3018, 1992.

AT Stopeck, AC Nicholson, FP Mancini, DP Hajjar. Cytokine regulations of LDL receptor gene transcription in Hep G2 cells. Submitted to the Journal of Biological Chemistry. February 1993.

ABSTRACTS:

M Josef, N Ng-Gray, A T Stopeck, and RW Rees-Jones. Decreased insulin receptor rhosphorylation in fibroblasts overexpressing a c-H-RAS1 cDNA. Clin.Res. Vol. 37, pp 859A, 1989. Presented at the Eastern regional AFCR meeting, October 1989.

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AT Stopeck, FP Mancini, AC Nicholson, DP Hajjar. Cytokine regulation of LDL receptor transcription. Presented at the national meeting of the American Society for Biochemistry and Molecular Biology June 1993.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Evan Charles Unger	Director, CT & MRI	6/15/54

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of California, Berkeley, CA	B.A.	1977	Economics
University of California, San Francisco School of Medicine, San Francisco, CA	M.D.	1982	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Internship 1982-1983	Dept. of Internal Medicine, University of Colorado Affiliated Hospitals, Denver, Colorado
Residency 1983-1986	Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri
Employment 1986-1988	Chief, Magnetic Resonance Imaging, Fox Chase Cancer Center, Philadelphia, Pennsylvania
1988-present	Director, CT & MRI, Associate Professor, Dept. of Radiology, University of Arizona, Tucson, Arizona
Certifications	National Board of Medical Examiners American Board of Radiology
Honors	Departmental Honors in Economics, University of California, Berkeley Alpha Omega Alpha Woodland Memorial Scholarship Summa Cum Laude Scientific Poster Session, SMRI
Professional Societies	Philadelphia Roentgen Ray Society American Roentgen Ray Society American College of Radiology Society for Magnetic Resonance Imaging Radiologic Society of North America Society for Magnetic Resonance in Medicine Association of University Radiologists Society of Cardiovascular Interventional Radiology

Academic Appointments

1986-1988	Assistant Clinical Professor, Department of Radiology, Temple University, Philadelphia, Pennsylvania
1988-present	Associate Professor, Department of Radiology, University of Arizona, Tucson, Arizona joint appointment at Arizona Cancer Center
1990-present	Director, Body Imaging Fellowship Training Program, Department of Radiology, University of Arizona, Tucson, Arizona

SELECTED PUBLICATIONS

1. Unger E, Littlefield J, Gado M. Water Content and Water Structure in CT and MRI Signal Changes Possible Influence in Detection of Early Stroke. Am J Neuroradiol 1988;9(4):687-692.
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15. Unger E, Shen DK, Fritz T. Status of Liposomes as MR Contrast Agents. JMRI 1993;3:195-198.
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2. Unger EC, Gatenby RA, Hersh E, Hynynen K, Jarrell B, Lund PJ, Modiano M, Putnam C. Work-In-Progress: CT-Guided Percutaneous Ethanol Injection Treatment of Liver Tumors. To be submitted.
3. Unger E, Fritz T, Shen DK, Lund P, Sahn D, Ramaswami R, Matsunaga T, Yellowhair D, Kulik B. Gas Filled Lipid Bilayers as Imaging Contrast Agents. To be submitted.
4. Unger E, Fritz T, Wu G, Shen D, New T, Wilke N, Kulik B. Liposomes as MR Contrast Agents. To be submitted.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
James A. Warneke, M.D.	Assistant Professor of Surgery

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of California, Riverside, CA	B.S.	1979	Biomedical Science
University of California, Riverside, CA	M.D.	1982	General Surgery
University of Arizona, Tucson, AZ	Internship	1983	General Surgery
University of Arizona, Tucson, AZ	Residency	1987	General Surgery
Rosewell Park Cancer Institute, Buffalo, NY	Fellowship	1989	General Surgery

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

ACADEMIC AND PROFESSIONAL APPOINTMENTS:

Department of Soft Tissue Sarcoma and Melanoma
Roswell Park Cancer Institute, Buffalo, NY, July 1989-December 1990

Department of Breast Surgery, Assistant Professor of Surgery,
Roswell Park Cancer Institute, Buffalo, NY, January 1991-October 1991

Arizona Health Sciences Center, Assistant Professor, Department of Surgery,
University of Arizona, Tucson, AZ, November 1991-present.

SOCIETIES:

American College of Surgeons
American Medical Association

PUBLICATIONS:

Warneke, James A., Petrelli, Nicholas, Herrera, Lemuel, et al: The Accuracy of Colonoscopy for the Detection of Colorectal Polyps. Dis Colon Rectum 1992;35:981-985.

Rappaport, William D. and Warneke, James A.: Subhepatic Appendicitis. American Family Practice 39:6, 1989.

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Villar, Hugo V., Warneke, James A., Peck, Michael, et al: Role of Surgical Treatment in the Management of Complications of the Gastrointestinal Tract in patients with Leukemia. Surgery, Gynecology and Obstetrics 165:217, 1987.

McIntyre, Kenneth D., Jr., Warneke, James A., Malone, James et al: Early Experience with and Inplantable Reservoir for Intravenous Chemotherapy. Arizona Medicine 42:306, 1985.

Putnam, Charles W., Buckley, Arthur, Warneke, James A., et al: the Mechanism of Hepatoprotection by Epsilon Aminocaproic Acid and Putrescine. Surgery 96:214, 1984.

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Warneke, James A., McKeating, John and Holyoke, Douglas: Survival of Stage III Breast Cancer after Postoperative Adjuvant Chemotherapy.

Sanchez-Forgach, E.R., Mamorinas, E.P., Warneke, James A., et al: Factors Affecting Outcome in Locally-Advanced Breast Cancer. Submitted to Surgical Oncology for publication.

Warneke, James A., Verazin, G.T., Driscoll, D.L., Karakousis, C.P., Takita, H.: Resection of Lung Metastasis from Soft Tissue Sarcomas: A Multivariate Analysis. Arch Surg. 1992;127:1407-1411.

Warneke, James A., Hunter, T.: Surgical Devices in Foreign Bodies, in Radiographic Guide to medical Devices and Foreign.

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HUMAN GENE TRANSFER PROTOCOL

9403-031/9209-031 Roth, Jack A., The University of Texas MD Anderson Cancer Center, Houston, Texas; *Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer NSCLC).*

9209-031/ RAC Approval of Original Protocol: 9-15-92/RAC
Approval of Original Protocol Withdrawn 12-3-93
9403-031/ RAC Approval of Resubmitted Protocol: 3-4-94/NIH

ABSTRACT
"Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)", Jack A. Roth, M.D., Study Chairman
The objective of this protocol is to evaluate the toxicity and possible therapeutic efficacy of the intralesional administration of retroviral constructs containing antisense (AS) K-*ras* (for tumors with mutated K-*ras*) and wildtype *p53* (wt*p53*) (for tumors with mutated or deleted *p53*) into residual endobronchial NSCLC which obstructs a bronchus and which is refractory to conventional therapy.

PATIENT ELIGIBILITY
1) Patients must have histologic proof of non-small cell lung cancer. Patients must be either unresectable, unable to receive primary external beam radiation therapy, or have failed primary external beam radiation therapy.
2) Patients must have an endobronchial tumor accessible by the bronchoscope. There must be some clinical evidence of bronchial obstruction.
3) All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).
4) All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.
5) A tumor biopsy must show either a K-ras mutation or a p53 mutation by single-strand conformation analysis.

TREATMENT PLAN
1) Patients will undergo bronchoscopy to assess the degree of obstruction. As much gross tumor as possible will be resected endoscopically. Patients may also have had brachytherapy as a tumor reduction modality.
2) Patients will undergo bronchoscopy under topical or general anesthesia. A Stifcor™ transbronchial aspiration needle (21g) will be passed through the biopsy channel of the bronchoscope. Tumor will be debulked endoscopically. The residual tumor site will be injected with 10^7 CFU/ml of the appropriate retroviral supernatant. The volume will be no greater than 10 ml. Injections will be circumferential and will be intratumor and submucosal. The AS-K-*ras* supernatant will be used for K-*ras* mutations and the *p53* supernatant will be used for *p53* mutations. The injections will be repeated daily for five consecutive days. The treatment will be repeated monthly.

PRETREATMENT EVALUATION
Pretreatment evaluation will consist of a complete history and physical including performance status, weight loss, description of previous and current malignant and non-malignant diseases and their treatment, and residual toxicities; location and size of the endobronchial lesion; chest x-ray, and laboratory studies including: quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, and urinalysis.
Prior to each course of therapy, the following clinical data will be ascertained and recorded:
1. CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray.
2. Measurement and bronchoscopic photograph of tumor.
3. All relevant information regarding drug dosage, tumor response, laboratory examinations, and treatment-related toxicities.

The primary endpoint of the study will be regrowth of the tumor. The effect of therapy in this group of patients will also be measured by determining length of patient survival, length of time the affected lobe of the lung remains aerated, and reduction in measurable endobronchial tumor. A maximum of 14 patients will be recruited in this study.

Protocol Title: *"Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)"*

Principal Investigator: Jack A. Roth, M.D.

The purpose of this protocol is to study a way of stopping cancer causing genes from functioning in human lung cancer cells in patients. Two types of cancer causing genes have been identified in human lung cancer. One of these genes is the K-ras oncogene. A mutation in a single base causes this gene to produce an abnormal protein which changes the cell from normal to malignant. A second gene that is altered in human lung cancer is the p53 tumor suppressor gene. A mutation or deletion of both copies of this gene causes the cell to become cancerous.

This protocol uses a gene therapy technique to turn off production of the abnormal K-ras protein and to replace the normal function of the p53 gene. DNA that produces "antisense" (mirror image) RNA is introduced into the cancer cell. This antisense RNA is specifically targeted to the RNA (sense) that is producing the abnormal K-ras protein. The sense and antisense RNAs bind together and this prevents the abnormal K-ras protein from being produced. The normal p53 gene will be introduced into lung cancer cells. This normal gene will overcome the function of the mutant gene and also replace the normal p53 function.

A novel feature of this protocol is that the genes will be introduced into human cancer cells in the patient by a retrovirus. A special retrovirus that is harmless to patients and rendered incapable of dividing is used. The antisense K-ras and the p53 gene are each inserted separately in this retrovirus. The retrovirus is taken up by the cancer cell and integrated into the cancer cell's DNA where the newly inserted gene is now expressed. Constant expression of these genes renders the cancer cell incapable of growing and dividing. Only dividing cells such as cancer cells can incorporate the retrovirus. Thus, this therapy is very selective for cancer cells. The antisense K-ras gene and the normal p53 gene are not harmful to normal cells. The retrovirus will be injected directly into the lung cancer.

Patients with lung cancer who are unable to have surgery, radiation therapy, or chemotherapy may participate. Patients must have a cancer that can be reached with the bronchoscope and that is obstructing air passage to the lung. Patients will have some of the tumor removed. The tumor will be tested to determine if it has one or the other genetic abnormality. The retrovirus capable of correcting the specific gene abnormality will be directly injected into the tumor. Following this the growth of the tumor will be measured. Patients may have multiple injections of the retrovirus. The tumor will also be studied to determine if the tumor cells are taking up the retrovirus.

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Protocol Title: *"Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)"*

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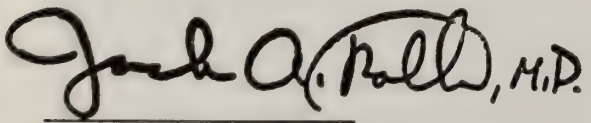
Appendix A: Evaluation Before and During Therapy
Appendix B: Zubrod Scale of Performance Status
Appendix C: Toxicity Criteria
Appendix D: Informed Consent

STUDY CHAIRMAN:



Jack A. Roth, M.D.
Department of Thoracic Surgery

STUDY COORDINATORS: Departments of Thoracic and Cardiovascular Surgery, Thoracic/Head and Neck Oncology, and Radiation Therapy.

M.S. McMurtrey, M.D.

C.F. Mountain, M.D.

J.B. Putnam, Jr., M.D.

G.L. Walsh, M.D.

F.D. Martin, R.N.

Tapas Mukhopadhyay, Ph.D.

DeWei Cai, M.D.

Jonathan C. Nesbitt, M.D.

W.K. Hong, M.D.
Dept of Thoracic/Head & Neck Oncology

R. Komaki, M.D.
Department of Radiotherapy

J. Lee, Ph.D.
Department of Biomathematics

PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title)

"Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)"

Study Chairman: **Jack A. Roth, M.D.**

Patient Eligibility:

Patients must have histologic proof of non-small cell lung cancer. Patients must be either unresectable, unable to receive primary external beam radiation therapy, or have failed primary external beam radiation therapy.

Patients must have an endobronchial tumor accessible by the bronchoscope. There must be some clinical evidence of bronchial obstruction.

All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).

All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.

A tumor biopsy must show either a K-ras mutation or a p53 mutation by single-strand conformation analysis.

Treatment Plan: (Include dose adjustment)

Patients will undergo bronchoscopy to assess the degree of obstruction. As much gross tumor as possible will be resected endoscopically. Patients may also have had brachytherapy as a tumor reduction modality.

Patients will undergo bronchoscopy under topical or general anesthesia. A Stifcor™ transbronchial aspiration needle (21g) will be passed through the biopsy channel of the bronchoscope. The residual tumor site will be injected with 10^7 CFU of the appropriate retroviral supernatant. The volume will be no greater than 10 ml. Injections will be circumferential and will be intratumor and submucosal. The AS-K-*ras* supernatant will be used for K-*ras* mutations and the *p53* supernatant will be used for *p53* mutations. The injections will be repeated daily for five consecutive days. The treatment will be repeated monthly.

Pretreatment and Treatment Evaluation:

Pretreatment evaluation will consist of a complete history and physical including performance status, weight loss, description of previous and current malignant and non-malignant diseases and their treatment, and residual toxicities; location and size of the endobronchial lesion; chest x-ray, and laboratory studies including: quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, and urinalysis.

Prior to each course of therapy, the following clinical data will be ascertained and recorded:

1. CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray.
2. Measurement and bronchoscopic photograph of tumor.
3. All relevant information regarding drug dosage, tumor response, laboratory examinations, and treatment-related toxicities.

Miscellaneous Information:

Not applicable.

Statistical Considerations:

The primary endpoint of the study will be regrowth of the tumor. The effect of therapy in this group of patients will also be measured by determining length of patient survival, length of time the affected lobe of the lung remains aerated, and reduction in measurable endobronchial tumor. A maximum of 14 patients will be recruited in this study.

Objectives:

The objective of this protocol is to evaluate the toxicity and possible therapeutic efficacy of the intralesional administration of retroviral constructs containing antisense (AS) K-*ras* (for tumors with mutated K-*ras*) and wildtype *p53* (wt*p53*) (for tumors with mutated or deleted *p53*) into residual endobronchial NSCLC which obstructs a bronchus and which is refractory to conventional therapy.

"Clinical Protocol Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)"

1.0 OBJECTIVES

The objective of this protocol is to evaluate the toxicity and possible therapeutic efficacy of the intralesional administration of retroviral constructs containing antisense (AS) K-ras (for tumors with mutated K-ras) and wildtype p53 (wtp53) (for tumors with mutated or deleted p53) into residual endobronchial NSCLC which obstructs a bronchus and which is refractory to conventional therapy.

2.0 BACKGROUND AND RATIONALE

2.1 Molecular events in NSCLC

Lung cancer remains the leading cause of cancer deaths in the United States where it kills more than 140,000 people annually. Recently, age-adjusted mortality from lung cancer has surpassed that from breast cancer in women. Although implementation of smoking-reduction programs has decreased the prevalence of smoking, lung cancer mortality rates will remain high well into the 21st century[1]. Unfortunately, all current treatment modalities, including radiation therapy, surgery, and chemotherapy, have limited effectiveness. The rational development of new therapies for lung cancer will depend on an understanding of the biology of lung cancer at the molecular level. Research in our laboratory has identified critical molecular events leading to NSCLC development and progression. The goal of this research is to directly modify the cancer cell to eliminate the expression of gene products which are responsible for the maintenance or progression of the malignant phenotype or to restore in normal form deleted or mutated gene products that suppress the characteristics of the malignant phenotype.

The purpose of this protocol is to investigate molecular mechanisms that may influence the growth and progression of human lung cancer; our goal is development of therapeutic agents specifically targeted at the molecular level. The most common lung cancer histologies (80%) are grouped under the term non-small-cell lung cancer (NSCLC) and include squamous, adenocarcinoma, and large-cell undifferentiated. Many of the current data on the molecular biology of lung cancer come from the study of the more uncommon small-cell lung cancer (SCLC). SCLC can be distinguished from NSCLC by the neuroendocrine features of the cells; SCLC is very responsive to chemotherapy but recurs rapidly after treatment. NSCLC also may serve as a model for other carcinogen-induced malignancies. The approaches and observations developed in this study may be applicable to other types of epithelial cancers.

Abundant evidence has accumulated that the process of malignant transformation is mediated by a genetic paradigm[2]. The major lesions detected in cancer cells occur in dominant oncogenes and tumor suppressor genes. Dominant oncogenes have alterations in a class of genes called proto-oncogenes, which participate in critical normal cell functions, including signal transduction and transcription. Primary modifications in the dominant oncogenes that confer the ability to transform include point mutations, translocations, rearrangements, and amplification. Tumor suppressor genes appear to require homozygous loss of function, by mutation, deletion, or a combination of these for transformation to occur. Some tumor suppressor genes appear to play a role in the governance of proliferation by regulation of transcription. It is possible that modification of the expression of dominant and tumor suppressor oncogenes may influence certain characteristics of cells that contribute to the malignant phenotype.

Despite increasing knowledge of the mechanisms involved in oncogene-mediated transformation, little progress has occurred in developing therapeutic strategies that specifically target oncogenes and their products. Initially, research in this area was focused on dominant oncogenes, as these were the first to be characterized. DNA-mediated gene transfer studies showed acquisition of the malignant phenotype by normal cells following the transfer of DNA from malignant human tumors. Activated oncogenes of the ras family were identified by this technique with transfection of human DNA into mouse NIH 3T3 cells.

Oncogene Mutations in Lung Cancer

Activation of the K-ras oncogene occurs in human NSCLC[3,4]. Recent studies using the polymerase chain reaction (PCR) and specific oligonucleotide hybridization show that a third of NSCLC patients have ras family mutations[5,6]. However, Reynolds and coworkers, using a sensitive NIH 3T3 cotransfection-nude mouse tumorigenicity assay, found that 12 of 14 (86%) lung tumor DNAs from smokers contained activated proto-oncogenes related to the ras family[7]. K-ras mutations occur primarily in adenocarcinomas, and the K-ras proto-oncogene has a point mutation in 30% to 40% of adenocarcinomas of the lung[5,6]. Thus, a minimum of 32,000 patients per year are expected to develop ras-mutation-positive lung cancer. K-ras mutations are associated with a history of tobacco consumption and may contribute to tumor progression.

The p53 gene is the most frequently mutated gene yet identified in human cancers. It is mutated in over 50% of human NSCLC[8]. The p53 gene encodes a 375-amino-acid phosphoprotein that can form complexes with host proteins such as large-T antigen and E1B[8]. Missense mutations are common for

the p53 gene and are essential for the transforming ability of the oncogene. The wildtype p53 gene may directly suppress uncontrolled cell growth or indirectly activate genes that suppress this growth. Thus, absence or inactivation of wildtype p53 may contribute to transformation. However, some studies indicate that the presence of mutant p53 may be necessary for full expression of the transforming potential of the gene. Mutations of p53 are common in a wide spectrum of tumors[10-12]; they occur in both NSCLC and SCLC cell lines and fresh tumors[13,14].

Options for specific targeting of oncogenes include inhibition of expression of a dominant gene or replacement of a deleted or mutated tumor suppressor gene. Progress in the understanding of the critical genes involved in tumor development and in technology for altering gene expression logically led to our studies of techniques for achieving these options. Initially, a model for specific inhibition of K-ras was developed. We chose to work with K-ras because of the applicability of the findings to a large number of tumors, because of our previous work with K-ras, and because information on the genetic organization and sequence of the ras gene family was readily available. Advances in antisense and retroviral gene transfer technology suggested that application of these techniques may mediate specific inhibition of oncogene expression.

Antisense mRNA, which is precisely complementary to the corresponding sense mRNA, inhibits translation. The mechanisms for this inhibition have not been completely defined but include inhibition of translation by ribosomes, degradation of sense-antisense duplexes by enzymes, and failure of export from the nucleus. Thus, specific targeting of a gene in a multigene family could occur if it possessed unique sequences in a region amenable to antisense inhibition, such as an initiation codon or splice site.

The working hypothesis that we developed is that reversal of a single altered genetic event in the cancer cell can potentially reverse critical features of the malignant phenotype of that cell. This finding has important therapeutic implications. Cancer cells have multiple genetic alterations. Therapy directed toward oncogenes will be practical only if therapeutic effects occur with targeting of one or two genes. It is unlikely that any therapy targeting oncogenes or their products will be absolutely specific for cancer cells. If other genes can compensate for loss of normal function by a specific oncogene mediated by an antisense construct, the harmful effects of the therapy will be reduced. Studies from our laboratory indicate that reversal of a single genetic alteration has profound effects on the growth and tumorigenicity of lung cancer cells[15,16]. Additional support for this concept comes from a recent study by Soriano and coworkers[17] in which transgenic mice were created that lacked a functional c-src proto-oncogene. The resulting developmental defect in the mice was osteopetrosis. The ubiquity of c-src, its high degree of conservation among species, and its role in mitosis suggest that inactivation would be lethal, but this was not the case; viable mice were recovered. A possible explanation is that other closely related nonreceptor tyrosine kinases such as yes and fyn can compensate for loss of c-src. Introduction of a single copy of a wildtype tumor suppressor gene into normal cells would be unlikely to have adverse effects if it occurred during therapy directed at replacing inactivated tumor suppressor genes in cancer cells.

Preliminary data on transfection of an antisense K-ras expression vector indicated that inhibition of expression of a single oncogene reduced the growth rate of cancer cells and tumorigenicity in nu/nu mice. However, transfected cells retained viability, as did cells with no endogenous K-ras mutation that were also transfected with the construct. The wtp53 appears dominant over the mutant gene and will select against proliferation when transfected into cells with the mutant gene[16,18]. Normal expression of the transfected wtp53 does not affect the growth of cells with endogenous wtp53. Thus, such constructs might be taken up by normal cells without adverse effects. This protocol focuses regional delivery of the two gene constructs, antisense K-ras and wtp53, to lung cancer cells in patients with unresectable obstructing endobronchial cancers. The efficiency of delivery and gene expression will be evaluated both in lung cancer cells and in normal cells in vivo. This is of importance for the design of constructs that may be useful therapeutically. The effects of these constructs on clinical progression of the cancer will be studied.

These approaches may lead to cancer therapy based on direct alteration of gene expression in cancer cells. Current therapy relies on attempts to kill or remove the last cancer cell. However, tumor cell dormancy is an established phenomenon making effective killing highly unlikely. Although inhibition of expression of some oncogenes may be lethal to the cancer cell, in some cases cell replication will slow or cease, thus rendering these cancers clinically dormant. Even if absolute specificity is not achieved, single oncogenes may still be important targets, because it is likely that adverse effects to normal cells will be minimal.

2.2 Natural history of locally unresectable NSCLC

Patients with NSCLC will die of their cancer in 86% of cases. Regional delivery of gene constructs to areas at risk for development of cancer has important implications for both prevention and therapy. Failure of therapy at the primary tumor site is a significant problem[19,20]. Of the 161,000 patients newly diagnosed with lung cancer in 1991, 45,080 will undergo surgical resection. Local recurrence as the first site of failure will occur in 9,000 of those patients.

Of the remaining patients, 52% will have localized tumors. However, 38% of these patients will have local failures following radiation therapy (22,900). Thus, 31,900 patients per year could benefit from improved local-regional therapy. Patients with unresectable obstructing NSCLC that is resistant to radiation therapy or who have coexisting metastases have a median survival of 6 months or less[21]. The Department of Thoracic Surgery at the University of Texas M. D. Anderson Cancer Center has extensive experience in the treatment of lung cancer. Over 1200 patients with lung cancer are seen yearly and over 200 of these patients undergo resection.

2.2.1 Measure of disease activity

The goal of this therapy is to halt or reverse the manifestations of the disease. The efficacy of therapy in this group of patients will be measured by determining length of patient survival, length of time the affected lobe of the lung remains aerated, and reduction in measurable endobronchial tumor. There is no curative therapy for this stage of disease and thus the outcome is predictable enough to allow for an assessment of the results of gene therapy. The measurements that will be used are described in Section 7.0.

2.2.2 Anticipated effect of protocol treatment

It is anticipated that the uptake of the retroviral constructs by proliferating NSCLC cells will decrease the rate of proliferation of these cells. This would increase the length of time the affected lung would remain expanded, prevent regrowth of the endobronchial tumor, and prolong the patient's survival.

2.2.3 Alternative therapies

Patients with unresectable endobronchial tumor recurrence that is partially or completely obstructing the airway and that have failed or are unable to receive external beam radiotherapy will be considered for this protocol. Existing therapies for this condition offer only short-term palliation. Most patients have recurred despite external beam radiotherapy. It may be possible to insert a brachytherapy catheter and administer additional radiotherapy. Patients receiving this treatment have a median survival of 6 months[21]. Patients failing brachytherapy would also be eligible to receive gene therapy. Tumor can be removed from the airway with the laser or biopsy forceps. This can be done in conjunction with injection of the retroviral construct thus decreasing the volume that must be injected. The administration of the retroviral constructs would not preclude the patient from receiving other palliative therapy if the tumor progresses.

2.3 Structure and characteristics of the biological system

2.3.1 Downregulation of activated K-ras expression with an antisense construct

2.3.1.1 Preliminary studies with plasmid DNA

The ras p21 gene product plays an important role in the intracellular signaling pathway of cells. Dysregulation of this pathway because of point mutations in regions critical to ras function confer transforming properties to the mutant protein. These mutations render the p21 protein gene product constitutively active by keeping the protein in the GTP-bound state. We began investigations on alterations of expression of p21[22]. A homozygous mutation at codon 61 was detected in the H460a large-cell undifferentiated NSCLC cell line clone; a normal glutamine residue (CAA) was substituted by histidine (CAT) using hybridization with specific oligonucleotide probes. Direct PCR DNA sequencing confirmed this. An antisense K-ras RNA construct selectively blocked the production of mutant p21 so the contribution of the mutated p21 protein to the malignant phenotype could be studied. A recombinant plasmid clone was constructed using a normal wildtype 2-Kb K-ras genomic DNA segment carrying second and third exons with flanking intron sequences subcloned into an Apr-1-neo expression vector in antisense orientation. The intron sequence used has a low degree of homology with other ras genomic sequences; it allowed specific inhibition of K-ras while preserving H-ras and N-ras expression. For example, the 241 bases flanking exon 2 and the 240 bases flanking exon 3 have no significant homology between K-ras and H-ras sequences. Previous studies of uptake of ras antisense oligonucleotides by cancer cells resulted in cell death instead of regulated growth, probably because functioning p21 is necessary for cell viability, and the oligonucleotides unselectively blocked p21 expression[23]. Unselective blockade of oncogene expression can therefore be harmful to both normal and cancer cells. An additional novel feature of this construct was the use of a β-actin promoter that can constitutively direct synthesis of RNA in a human tumor cell. The 2-Kb DNA insert was stably integrated into H460a cells, as shown by Southern hybridization. Northern blot analysis detected expression of antisense RNA. Western blot analysis showed a

95% reduction in K-ras p21 protein synthesis in the clones expressing the antisense RNA, whereas H460a cells and sense K-ras clones showed unchanged levels of K-ras p21 protein. Total p21 detected with a pan-ras monoclonal antibody showed only a slight decrease in the antisense clones, suggesting other ras genes were not affected. To confirm this, expression of ras genes was measured by cDNA PCR. The cDNA synthesized from the total RNA was subjected to PCR amplification using amplimers corresponding to the 5'-end of the first exon and the 3'-end of the second exon. Because the antisense RNA was generated from the second and third exons of K-ras, PCR-amplified cDNA represented the level of endogenous K-ras mRNA. N- and H-ras-specific oligonucleotides were used to determine expression of their respective genes. A 118-bp segment of endogenous p53 was co-amplified in the same reaction mixture as an internal PCR control.

Cells expressing antisense RNA showed complete inhibition of K-ras mRNA synthesis. There was no change in H-ras or N-ras expression in either antisense or sense transfectants. Antisense transfectants showed a 3-fold reduction in growth rate compared to sense transfectants and parental H460a cells but continued to grow in culture. Expression of antisense K-ras RNA reduced the growth rate of H460a tumors in nu/nu mice. Tumorigenicity of cell lines expressing antisense RNA was assessed by subcutaneous injection of 10^5 cells in nu/nu mice. Unmodified H460a cells formed tumors in all mice in 15 days. No tumors developed in mice injected with H460a antisense cells during 120 days of observation, whereas H460a cells transfected with Apr-1-neo sense plasmid formed tumors similar to those formed by unmodified H460a cells. These experiments show that in H460a cells engineered to synthesize antisense K-ras RNA, the levels of K-ras mRNA and K-ras p21 protein were dramatically reduced. Our studies show that a construct can be made that distinguishes among members of the ras family. Previous studies with ras AS oligonucleotides showed inhibition of total p21 expression which led to cell death[23], whereas our data show that antisense RNA generated from the genomic DNA of the K-ras gene can specifically inhibit K-ras expression. Inhibition of K-ras reduced the growth rate of H460a cells but did not alter cell viability or continued growth in culture, which suggests that redundancy in p21 expression may compensate for absence of expression by one member of this family so that functions essential for maintenance of cell viability are preserved. When antisense K-ras was transfected into H322a cells, which are homozygous for wtK-ras, the cells were unchanged in viability or rate of growth. These studies provide evidence that reduction in the expression of a single mutant gene product can reduce tumor cell proliferation and tumorigenicity.

2.3.1.2 Gene construct

The retroviral vector construct contains the AS-K-ras fragment with its β-actin promoter inserted into the LNSX vector[24,25]. The orientation of the insert is such that the transcription of the AS-K-ras is driven by the β-actin promoter downstream from the insert. A minor modification eliminating the SV40 promoter may also be used.

2.3.1.3 Packaging

Because recombination events may lead to the production of a replication-competent virus, a safe and efficient amphotropic packaging cell line is necessary for transfer of exogenous genes into human cancer cells. We used a packaging cell line constructed so the gag-pol and env genes are separated on two different plasmids[26]. For this protocol retroviral supernates from the GP+envAm12 packaging cell line will be used. This packaging cell line has received prior approval for use in human gene therapy clinical trials. The presence of functioning retroviral genes in the packaging cell line will be monitored by an assay for reverse transcriptase production and by immunoprecipitation of env protein by metabolic labeling and immunoprecipitation with anti-env antiserum[26]. Continued absence of infectious virus will be determined from transfection-infection experiments. A neo-containing vector will be transfected into GP+envAM12 cells; colonies will be selected with G418. The supernate will be used to infect NIH 3T3 cells. Selection with G418 will be done after one month to ensure the survival of rare recombinants that do not have the neo gene but subsequently infect neo-positive cells. Supernate from the infected NIH 3T3 cells should not be infectious. These secondary supernates will be used to infect naive NIH 3T3 cells. Lack of infectivity will indicate absence of replication competent virus. Previous human studies have used the GP+envAm12 producer cell line. We will use this cell because of the extensive experience with its use and prior approval for human use. Supernate from the packaging cells will be produced in

serum-free medium. Clones will be established and lots of frozen supernate prepared. This packaging cell line was used in all preclinical studies.

2.3.1.4 Preclinical studies

The 2 Kb K-ras fragment (genomic exons 2 and 3) with a β-actin promoter was cloned into the LNSX retroviral vectors in both orientations. The p53 cDNA with its β-actin promoter was cloned into the LNSX retroviral vectors in both orientations. The LNSX-AS-K-ras was successfully packaged in the GP+envAm12 packaging cell line. Initial titers ranged up to 10^7. The constructs were then transduced into H460a cells. Specific expression of K-ras AS RNA was shown by slot blot analysis using vector only negative controls and a β-actin probe for a loading control. Western blotting studies showed that expression of the K-ras p21 protein was specifically reduced. Next the effect of multiple cycles of transduction on transduction efficiency was assessed. Transduction efficiency was assessed on a functional level (Fig. 1). H460a cells were transduced with either LNSX or LNSX-AS-K-ras daily for 4 consecutive days. Cells grew for 7 days without selection. The percent reduction in the growth fraction of the AS transduced cells reflects the efficiency of transduction as growth of a selected population of AS transduced cells does not occur during this time period. The growth of the unselected AS transduced cells was less than 20% at 7 days. Thus, the simple manipulation of exposing cells to the packaged retrovirus for 4 consecutive days caused a striking increase in transduction efficiency. In a subsequent experiment H460a cells were transduced daily for 7 consecutive days with LNSX-AS-K-ras and then selected for colony formation in G418 (Fig. 2). Colonies were compared to H460a cells that were not exposed to selective medium. Following selection the efficiency of colony formation by the transduced cells was 98%. This reinfection strategy is applicable to regional therapy. The low acute toxicity of the retroviral constructs should permit multiple treatments. Intratracheal and subcutaneous injections indicate that up to 3 injections can be tolerated in mice with no acute toxicity. It is anticipated that the residual number of endobronchial tumor cells can be reduced to $<10^7$ so that an excess ratio of retroviral particles to proliferating tumor cells can be achieved.

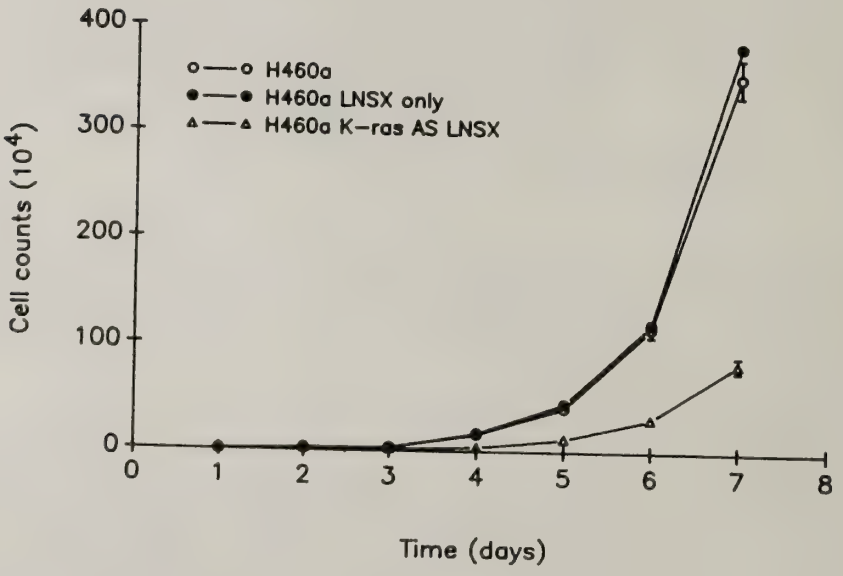


Figure 1: Functional transduction efficiency of LNSX-AS-K-ras in H460a cells. Growth curves are shown for 10^5 cells/well seeded in 12 well plates. H460a cells were infected by incubation 0.5 m of viral supernate stock from either LNSX or LNSX-AS-K-ras ($6X10^6$ CFU/ml) daily for 4 consecutive days in the presence of 8μg/ml of polybrene. The parental H460a cells served as a control. Cells were not selected with G418. Cells were counted daily. The mean ± SE is shown for 3 replicates.

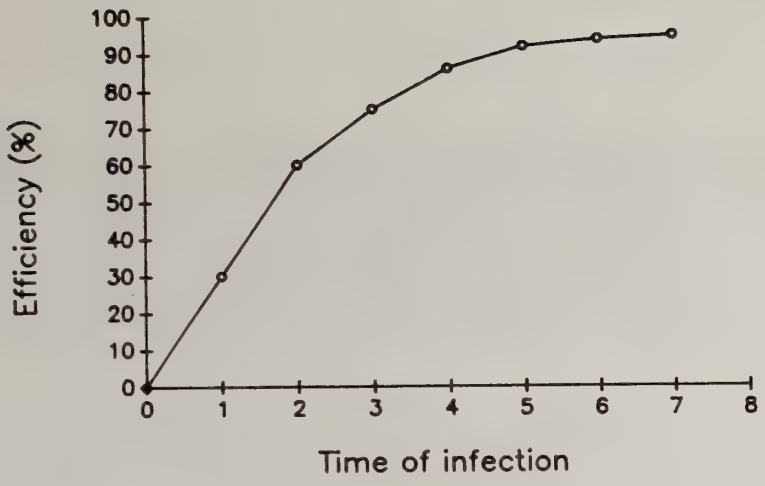


Figure 2: H460a cells were infected with LNSX-AS-K-ras by incubating 10^4 cells with 0.5 ml of viral stock ($6X10^6$ CFU/ml) produced by the packaging cell line GP+envAm12 in the presence of 8μg/ml of polybrene. Infection was done daily for 1 to 7 days. Two days later cells were plated in equal numbers into selective media containing 200μg/ml G418. Control H460a cells were plated at equal cell numbers to determine baseline colony forming efficiency. The infection efficiency was measured by determining the percent of the unselected colony number formed by the G418 selected colonies.

The tumorigenicity of the transduced H460a cells was studied in an orthotopic lung cancer model. Intratracheal inoculation of H460a cells in irradiated (350cGy) nu/nu mice resulted in the growth of endobronchial tumors with mediastinal extension in >80% of mice after 4 weeks. The H460a-AS-LNSX, H460a-LNSX, and H460a cells (10^5/mouse) were injected endotracheally in 0.1 ml of medium and the mediastinal block was harvested after 4 weeks. Mice were assessed for tumor growth without knowledge of the treatment group. Seven of 9 mice inoculated with H460a-LNSX (mean volume 12.5 ± 2.2 SE mm^3) and 12 of 14 mice inoculated with H460a parental cells (mean volume 39.9 ± 4.25 SE mm^3) had tumors. Only 3 of 17 mice receiving H460a-AS-LNSX cells had tumors (mean volume 2.95 ± 1.25 mm^3). We conclude that 1) retroviral gene transduction can be used to express anti-sense constructs in human tumor cells at levels that mediate a biologic effect; 2) AS-mediated inhibition of activated K-ras expression effectively inhibits proliferation and tumorigenicity of human cancer cells. Expression of the AS-LNSX expression in the H460a cells has been stable up to 6 months.

In a second experiment, H460a cells (10^5/mouse) were injected into the right mainstem bronchus via a tracheostomy incision. Three days later mice were inoculated endobronchially with 0.15 ml of retroviral supernate or control medium for 3 days. Tumor growth was suppressed in the group receiving supernate from LNSX-AS producer cells (Table 1). Histologic light microscopic study of 3 specimens from the LNSX-AS group did not reveal any residual H460a cells.

Table 1: Endotracheal inoculation of retroviral supernate for treatment of established (3 day) H460a human lung cancers growing endobronchially in nu/nu mice. The H460a cells were injected at 10^5 per mouse. The titer of the retroviral supernate was $5X10^4$ CFU/ml. Mice were injected with 0.15ml of supernate daily for 3 days.

Producer Cells	CFU/dose	No. of Tumors/ No. of Mice (%)	Mean Tumor Volume ± SE
H460a	0	7/8	(87.5%) 42.3 ± 7.9
LNSX	$7.5X10^5$	7/8	(87.5%) 44.9 ± 10.1
LNSX-AS	$7.5X10^5$	1/6	(16.7%) 7.7*

* Volume of the single tumor that occurred in this group

2.3.2 Restoration of expression of wtp53 gene product

2.3.2.1 Preliminary studies with plasmid DNA

The p53 gene is the most commonly altered gene yet described in human cancers. To

study this gene, a cell culture model system of cell lines varying in p53 expression was established. The H322a lung adenocarcinoma cell line expresses the mutant p53 protein as shown by the presence of high levels of endogenous p53 mRNA and phosphorylated protein. We showed that the H322a cell line has a G:T transversion at codon 248 (Arg to Leu) with absence of the wildtype allele. The H358a cell line has a homozygous p53 deletion. The H460a and H226b cell lines are homozygous for the wildtype p53. Expression vectors for sense (S-p53) and antisense p53 (AS-p53) cDNA with a β-actin promoter were constructed to study the effect of wtp53 expressed in lung cancer cells with mutant or deleted p53 and the effects of reducing wildtype and mutant p53 expression.[16]

Stable transfectants of p53 mutant cells (H322a) or deleted p53 (H358) expressing S-p53 could not be rescued. Failure to isolate colonies expressing sense p53 RNA in cells with homozygous mutant or deleted alleles shows that wtp53 can suppress transformation in cancer cells expressing a mutant p53 or having a homozygous p53 deletion.

In general, transfection with AS-p53 reduced colony formation (10-fold) by cells with endogenous mutant p53. This indicates that expression of mutant p53 contributes to the transformed phenotype. As expected, cells with wtp53 (H226b) showed increased tumorigenicity when transfected with AS-p53. The H226b cells expressing AS-p53 grow significantly more rapidly in nu/nu mice than the cells transfected with the control plasmid. This indicates that elimination of the wtp53 gene product enhances features of the malignant phenotype.

Our studies showed that wtp53 is dominant and can suppress the malignant phenotype in cells with mutant or deleted p53. The presence of the mutant p53 confers transforming potential to the gene product, which can be suppressed by AS-p53. Thus, in cancer cells both the absence of wtp53 and the presence of certain p53 mutations may enhance the malignant phenotype.

2.3.2.2 Gene construct

The retroviral vector construct contains p53 cDNA with its β-actin promoter inserted into the LNSX vector[24,25]. Preclinical studies were performed with this vector. Subsequently a new vector was developed which does not have the SV40 promoter. This is designated LNp53B and will be used in the clinical trials.

2.3.2.3 Packaging
See section 2.3.1.3.

2.3.2.4 Preclinical studies

The LNSX-p53 and the DC-p53 were transduced into H322a (mutant p53), H358a (deleted p53), and H460a (wt p53). H322a cells that underwent one cycle of infection with the wtp53 construct but without G418 selection had an over 3-fold reduction in proliferation compared to cells that received either the unmodified vector or no treatment. Two cycles of transduction without G418 selection resulted in a 5-fold reduction in proliferation (Fig. 3). A similar result was observed for the H358a cell line when transduced with LNSX-p53. The proliferation of the H460a cell line which has a wildtype p53 was not altered by transduction with any of the p53 retroviral constructs (Fig. 4). Thus, retroviral mediated gene transfer of wtp53 into human lung cancer cells with deleted or mutated p53 significantly reduces the proliferation of those cells. The expression of the mutated p53 protein is uniform in cultured cell lines as detected by immunohistochemistry. In fresh lung tumors that express high levels of p53 protein, expression is detected in >90% of cells.

A critical question is the ability of the retroviral constructs to transduce established tumor cells in vivo. This question was addressed by injecting H460a (10^5) cells in the mouse right mainstem bronchus followed 3 days later by lavage with LNSX retroviral supernate (10^6 CFU in 0.1 ml). LNSX was used so that the neo gene could be used as a marker for transduction. It was necessary to recover tumor cells for analysis so that the AS construct was not used. Tumors were harvested and the presence of the neo gene was assessed by Southern hybridization. The neo gene was detected in the DNA from the H460a cells indicating successful transduction of the retrovirus 30 days after lavage. Although this data is encouraging, the model has limitations. Direct injection of endobronchial tumor is not possible in this model. Other sites of direct injection do not accurately simulate the milieu of endobronchial lung cancer.

Thus, definitive answers concerning efficacy must be obtained through this clinical trial.

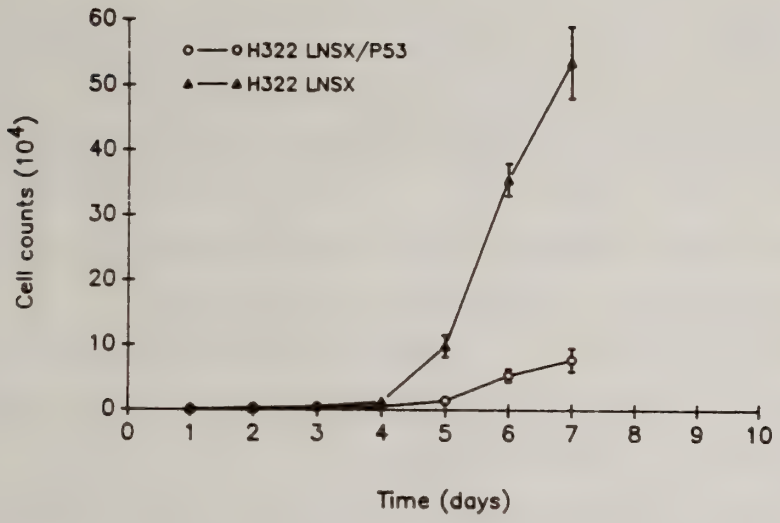


Figure 3: Growth curves are shown for 10^4 cells/well seeded in 12 well plates. H322a cells were infected by incubation 0.5 ml of viral supernate stock from either LNSX or LNSX-p53 (10^6 CFU/ml) on 2 consecutive days (beginning at day -2) in the presence of 8μg/ml of polybrene. Cells were not selected with G418. Cells were counted daily. The mean ± SE is shown for three replicates.

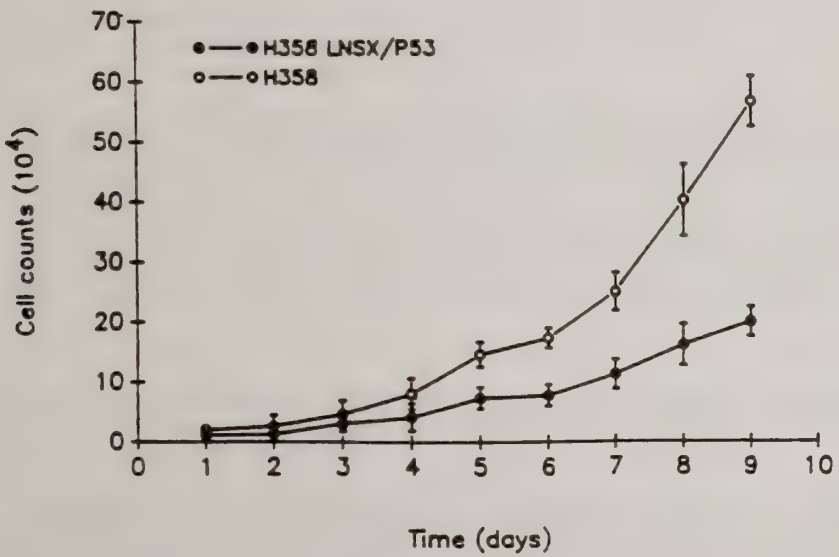


Figure 4: Growth curves are shown for 10^4 cells/well seeded in 12 well plates. H358a cells were infected by incubation 0.5 m of viral supernate stock from either LNSX or LNSX-p53 (10^6 CFU/ml) in the presence of 8μg/ml of polybrene. The parental H358a cells served as a control. Cells were not selected with G418. Cells were counted daily. The mean ± SE is shown for three replicates.

3.0 **SAFETY INFORMATION**

3.1 Continued absence of replication competent infectious virus was determined from transfection-infection experiments. A neo-containing vector was transfected into packaging cells; colonies were selected with G418. The supernate were used to infect NIH 3T3 cells. Selection with G418 will be done after both 72 hrs and one month to ensure the survival of rare recombinants that do not have the neo gene but subsequently infect neo-positive cells. Supernate from the infected NIH 3T3 cells should not be infectious. These secondary supernate were used to infect naive NIH 3T3 cells. Lack of infectivity indicates absence of replication competent virus. To date experiments have been negative. These studies and the following safety studies will be performed by Microbiological Associates, Inc.

3.2 Sterility will be assured by testing for aerobic and anaerobic bacteria, fungus, and mycoplasma.

3.3 S^+/L^- assay including 3T3 amplification must be negative.

3.4 PCR assay for the absence of 4070A envelope gene must be negative.

3.5 Reverse transcriptase assay must be negative.

4.0 **PATIENT ELIGIBILITY** Patient Age: 18 to 80

4.1 Patients must have histologic proof of non-small cell lung cancer. Patients must be either unresectable, unable to receive primary external beam radiation therapy, or have failed primary external beam radiation therapy. Patients must have bronchial obstruction as their major problem requiring therapy. Patients will be excluded or removed from the study if at any point they require systemic chemotherapy for control of progressive metastatic disease.

4.2 Patients must have an endobronchial tumor accessible by the bronchoscope. There must be some clinical evidence of bronchial obstruction.

4.3 All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).

4.4 All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.

4.5 A tumor biopsy must show either a K-ras mutation or a p53 mutation by single-strand conformation analysis[27]. Material obtained previously and embedded in paraffin may be analyzed. If a new biopsy is required, the patient will be entered into the protocol and informed consent obtained if protocol entry is the sole reason for the biopsy. K-ras mutations will be determined by specific oligonucleotide hybridization to PCR amplified tumor DNA. Mutations of the p53 gene will be determined by SSCP analysis of exons 5-8 of PCR amplified tumor DNA.

4.6 Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative.

5.0 **TREATMENT PLAN**

5.1 Patients will undergo bronchoscopy to assess the degree of obstruction. As much gross tumor as possible will be resected endoscopically. Patients may also have had brachytherapy as a tumor reduction modality.

5.2 Patients will undergo bronchoscopy under topical or general anesthesia. A Stifcor™ transbronchial aspiration needle (21g) will be passed through the biopsy channel of the bronchoscope. The residual tumor site will be injected with 10^7 CFU of the appropriate retroviral supernate. The volume will be no greater than 10 ml. Protamine will be added at a concentration of 5μg/ml. This is 0.2% of the amount given intravenously to reverse heparinization.
Injections will be circumferential and will be intratumor and submucosal. The AS-K-ras supernate (LNSX-AS-K-ras) will be used for K-ras mutations and the p53 supernate (LNp53B) will be used for p53 mutations. The injections will be repeated daily for five consecutive days. The treatment will be repeated monthly. Treatment will continue as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.

5.3 Patients will wear a surgical mask for 24 hours following injection of the retroviral supernate. All medical personnel will wear masks routinely during bronchoscopy and injection of the retroviral supernates. Anti-tussives will be prescribed as necessary. All patients will be kept in isolation during the time they are receiving injections of the retroviral supernatant and for

48 hours after the last injection.

6.0 PRE-TREATMENT EVALUATION

6.1 A complete history and physical to include performance status, recent weight loss, usual weight and concurrent non-malignant disease and its therapy, and all prior anticancer treatments must be recorded.

6.2 Laboratory studies shall include quantitative immunoglobulins; a CBX with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, urinalysis, and chest x-ray.

6.3 Any residual toxicity from prior therapies should be recorded using the grading schema in Appendix C.

6.4 Appropriate studies should be obtained to fully define the extent and severity of existing or suspected malignant and non-malignant disease.

6.5 The location and size of the endobronchial lesion must be recorded prior to treatment. A photograph of the area will be taken at a fixed distance from the lesion. The area will be measured in 2 dimensions (product of longest and perpendicular dimensions).

7.0 EVALUATION DURING STUDY

7.1 Patients will have a CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray prior to each course of therapy. Serum will be collected pre- and post-treatment for analysis of antibodies to retroviral proteins.

7.2 History and physical with performance status and weight should be recorded before each course.

7.3 The tumor will be photographed bronchoscopically at the beginning of each course. Tumor measurements are to be recorded before each course.

7.4 All relevant information regarding drug dosage, tumor response, laboratory examinations, and treatment-related toxicities must be recorded before each treatment is given.

7.5 Parameters to be Measured <u>In Vitro</u>

7.5.1 Bronchoscopic tumor and normal bronchial epithelial biopsies will be obtained prior to the beginning of each course. Tissue will be fixed immediately in 4% paraformaldehyde and 0.5% gluteraldehyde at 4°C. This will permit extraction of DNA and RNA and permit <u>in situ</u> hybridization.

7.5.2 Biopsies will be analyzed for incorporation of the transduced gene into the host genomic DNA and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction and by <u>in situ</u> hybridization.

7.5.3 All patients will be evaluable for response and toxicity following one course of therapy

7.6 An autopsy will be requested on all patients enrolled in the protocol who die. DNA will be extracted from tumor and normal tissues to determine if the retroviral vector is integrated. PCR amplification of specific sequences will be used to determine this.

7.7 Three blood samples will be collected at one-half hour intervals following injection of the retroviral supernate. These samples will provide leukocytes to analyze for integration of retroviral DNA. Serum will be tested for antibodies to retroviral envelope proteins. This will be done by western blot analysis performed by Microbiological Associates, Inc. (Rockville, MD). Patients will be tested monthly during treatment, monthly for the first three months following completion of treatment, every three months for the remainder of the year following completion of treatment, and then at least yearly thereafter.

7.8 Normal tissue samples will be collected during the follow-up visits and bronchoscopies. These will include samples of normal bronchial mucosa, leukocytes, and germ cells. These tissues will be analyzed for incorporation of the retrovirus.

8.0 CRITERIA FOR RESPONSE AND TOXICITY

8.1 The tumor bed will be photographed prior to each course of therapy.

8.2 The longest diameter and its perpendicular will be measured. Size will be reported as the product of the diameters.

8.3 The rate of regrowth of the tumor will be calculated from these measurements.

8.4 Time to Progression and Survival Duration: The time to progression will be measured from the first observation with reduction in tumor bulk until there is evidence of progressive disease. Progressive Disease is defined as an increase of $\geq$ 25% in the sum of the products of the diameters of the measured lesion. Patients must have received at least two courses of therapy before a designation of progression is made. The survival of patients will be measured from entry into protocol.

8.5 All toxicities encountered during the study will be evaluated according to the grading system (0-4) in Appendix C and recorded prior to each course of therapy. Duration of the toxicity and its treatment will be recorded. Life-threatening toxicities should be reported immediately to the Study Chairperson, who in turn, must notify the IRB, RAC, and FDA.

8.6 Potential risks of retroviral gene transduction

8.6.1 Insertional mutagenesis. The possibility of causing malignancy in normal cells secondary to random insertion of the retroviral vector in the genome exists although this risk is thought to be very low. Tests of viral supernate will be conducted to assure that no replication competent virus is present. Non-replicating bronchial epithelial cells do not take up the vector in the mouse studies.

8.6.2 Risk from murine retrovirus. The retrovirus derived from the Moloney murine leukemia virus is modified so that it no longer contains intact viral genes. Thus, it cannot produce an intact infectious virus. Assays will be performed on the retroviral vector supernate and the packaging cell to insure that replication competent virus is not present (see Section 3.0). Extensive safety studies have been performed on related retroviral constructs in primates. Large infusions of infectious murine amphotrophic virus produce no acute pathologic effects. Primates have also received retroviral gene-modified autologous bone marrow cells with no evidence of toxicity as long as 4 years after infusion[28].

8.6.3 Efficacy of aminoglycoside antibiotics. The neomycin resistance gene product, neomycin phosphotransferase, phosphorylates the 3' hydroxyl group of the aminohexose I of neomycin and its analogues. Amikacin, but not gentamicin and tobramycin which do not contain an hydroxyl at the 3" position, is inactivated. Thus, induction of the neomycin resistance gene would not exclude aminoglycosides or any other conventional antibiotic from use in these patients.

9.0 CRITERIA FOR DISCONTINUING THERAPY

9.1 Increasing endobronchial tumor (greater than 25% increase in product of perpendicular diameters) after a minimum of 2 or more courses of therapy.

9.2 The development of unacceptable toxicity defined as unpredictable, irreversible, or Grade 4. Patient refusal of therapy due to a specific toxicity should be graded as 4 and an explanatory note recorded.

9.3 Non-compliance by patient with protocol requirements.

9.4 Patient refusal to continue treatment.

9.5 Criteria for removal from protocol:
a) Refusal to continue study participation
b) Significant hemoptysis
c) Coagulopathy
d) Progressive postobstructive pneumonia

10.0 DATA AND PROTOCOL MANAGEMENT

10.1 Protocol Compliance: The attending physician and oncology research nurse must see patients prior to drug administration. All required interim and pretreatment data should be available and the physician must have a designation as to tumor response and toxicity grade.

10.2 Data Entry: Data must be entered into the Clinical Data Management System before a course of

therapy can be given. A brief explanation for missing data should be recorded as a comment.

10.3 Accuracy of Data Collection: The Study Chairperson will be the final arbiter of response of toxicity should a difference of opinion exist.

10.4 Statistical Considerations: A single arm study design will be used. It is assumed that the regrowth of the tumor occurs in 90% of patients (>25% increase in area). If the frequency of regrowth is reduced to 60% by the treatment, then using a one-sided test with 80% power and α=.05, a sample size of 14 patients is needed. It should be possible to accrue this number within 18 months.

To prevent enrolling more patients in the trial when excessive toxicity is found, a Bayesian early stopping rule will be implemented. The design is described as follows:

1) The toxicity is considered unacceptable at the level when more than one-third of the patients develop Grade 3 toxicity or when more than one-sixth of the patients develop Grade 4 toxicity.

2) The probability of toxicity is assumed to follows the beta distribution with a non-informative prior [Uniform (0,1)].

3) The trial will be terminated when the probability of toxicity is greater than the undesired level [defined in (1)] with a high confidence. In this design, we choose this confidence probability being 0.90.

Under the above specification, the trial will be stopped when the Grade 3 toxicity is observed in the following cases: (The number of toxicity is given in the numerator and the number of total cases in the denominator.) 2/2, 3/3, 3/4-5, 4/6-7, 5/8-9, 6/10-12, 7/13-14.

The trial can also be stopped when the Grade 4 toxicity is observed in: 1/1-2, 2/3-6, 3/7-10, 4/11-14.

11.0 REPORTING REQUIREMENTS

11.1 Any life-threatening and/or unexpected and serious (grade 3 or 4) toxicity will be reported immediately to the Study Chairperson who, in turn, must notify the Food and Drug Administration, Surveillance Committee, and the sponsoring agency.

11.2 The Protocol Data management System will be used for data collections.

12.0 REFERENCES

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THE UNIVERSITY OF TEXAS M. D. ANDERSON CANCER CENTER

INFORMED CONSENT

Protocol Title: **"Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer (NSCLC)"**

1. _____ _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so that you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other consents you may have signed.

DESCRIPTION OF RESEARCH

2. PURPOSE OF THE STUDY: This is a clinical research study to evaluate a new therapy. A type of virus found in mice (called murine retrovirus) has been modified to make it inactive so it will not cause human disease. It is capable of carrying a human gene that has been shown in laboratory experiments to inactivate or replace defective genes that cause lung cancer. There is also a bacterial gene that helps trace these genes. The retrovirus will be injected directly into lung cancer cells that are growing and obstructing the airway to the lung.

3. DESCRIPTION OF RESEARCH: It has been recognized that cancer is associated with abnormalities in certain genes (oncogenes) which can become mutated or lost during the process of cancer development. This study is an attempt to restore a pattern of normal behavior to the cancer cell by correcting one of these gene abnormalities.

   This experiment is designed as follows: Lung cancer patients whose tumors obstruct part of their tracheobronchial trees are eligible to be considered for this study. Tumors that are removed during regular bronchoscopic exam will be examined to determine whether they contain the specific genetic mutation we are attempting to treat.

   Fourteen patients whose tumors have this mutation will be enrolled in this study. Tumors must be reducible to a small amount with removal through the bronchoscope or laser treatment. If it is not possible to find this mutation in specimens from previous

biopsies, new biopsies must be performed. The residual tumor will be injected daily for 5 consecutive days with a retrovirus containing a gene that may overcome the mutation detected in the cancer cells. This treatment will be repeated monthly as long as there is evidence that the tumor is not growing.

4. **RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:**

A few additional biopsies will be required. A bronchoscopic biopsy samples a very tiny piece of the mucosa. Risks of biopsies include hemoptysis (coughing up blood) which is usually transient and self limited. Severe hemorrhage requiring emergency maneuvers including intubation and thoracotomy are exceedingly rare. Extremely rarely, experimental procedures result in unforeseen harm. Appropriate medical therapy will be made available for the treatment of such consequences.

4a. This clinical procedure may involve unforeseeable risks to the participant (or to the embryo or fetus, if the participant is or may become pregnant during treatment). To help prevent injury to unborn children, upon recommendation by the attending physician, the participants should practice adequate methods of birth control throughout the period of their involvement in this clinical research study.

4b. *Possibility of Causing a New Cancer*. The possibility of causing cancer in normal cells exists although this risk is thought to be very low. Tests of viral supernate will be conducted to assure that possible disease causing virus is not present in the material to be injected.

Risk from murine retrovirus. The retrovirus derived from the Moloney murine leukemia virus is modified so that it no longer contains intact viral genes. Thus it cannot produce an intact infectious virus. Therefore, the risk of getting a virus infection is extremely low.

4c. A small amount of protamine is added to the retroviral mixture. Protamine is normally used to help blood clot. In this case it is used to help the virus enter the cancer cell. In the clone used it will not affect blood clotting. Infrequent allergic reactions and low blood pressure have occurred as side-effects when this drug is given. These are very unlikely to occur with the small dose given.

5. POTENTIAL BENEFITS: There is potential therapeutic benefit from this procedure. It is possible that injection of the retrovirus will slow the growth of the tumor and prevent further blockage of the lung. This study will also form the basis for future studies which may be able to apply this technology to the treatment of earlier cancer.

6. ALTERNATE PROCEDURES OR TREATMENTS: The eligibility rule for this study limits subjects to people who have already been through conventional therapy (such as surgery or external beam radiation therapy), and those for whom no other therapy is feasible. Therefore, the only alternatives would be other clinical research studies or no specific antitumor treatment. Of course, standard supportive care will be given regardless of participation in therapy.

Permission for Autopsy: It is anticipated that permission to perform an autopsy will be requested in the event of a patient's death following participation in this study. Accurate determination of the cause of death is of vital importance to future patients.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the procedure involved and the investigator has been willing to reply to my inquiries. This procedure will be administered under the above numbered, titled and described clinical research protocol at this institution. I hereby authorize Dr. Jack A. Roth, the attending physician/investigator, and designated associates to administer the treatment.

8. I have been told and understand that my participation in this clinical research study is voluntary. I am free not to participate or to withdraw my consent and discontinue my participation at any time. Such action will not result in any penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

If I am considering not participating in this research or withdrawing my consent and stopping participation, I have been advised that I should first discuss with my physician the potential consequences of such a decision.

In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

I will be informed of any new findings developed during the course of this clinical research study which may relate to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration, or National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that, should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available for treatment at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for any injury that is not caused by negligence.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of the study, I understand that I may be required to cover the cost of subsequent doses.

    Costs related to my medical care that are not part of this research (including expensive tests or procedures such as bronchoscopy and biopsies) shall be my responsibility. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, new drugs, or possible patentable procedures, in which event I herein disclaim and hereby waive any right or claim to receive any compensation or benefits from the subsequent use of information acquired and developed through participation in this research project.

13. I understand that practicing effective contraception is medically necessary and a prerequisite for my participation in this clinical research study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study may be terminated at the sole discretion of the investigator.

14. I may discuss any questions or problems during or after this study with **Dr. Jack A. Roth at (713) 792-6932.** In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman or the Surveillance Committee at (713) 792-3220 and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based upon the above, I consent to participate in the research and have received a copy of the consent form.

DATE	**SIGNATURE OF PARTICIPANT**
WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR	**SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP**

I have discussed this clinical research study with the Participant and/or his or her authorized representative using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe the participant understood this explanation and has voluntarily agreed to participate.

PHYSICIAN/INVESTIGATOR

BIOGRAPHICAL SKETCH **CVs**

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Jack A. Roth, M.D.	Professor and Department Chairman

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Cornell Univ, Ithaca, NY	B.A.	1967	Economics
Johns Hopkins Univ Sch of Med, Baltimore	M.D.	1971	Medicine
UCLA Sch of Medicine, Los Angeles, CA	Fellowship	1971-1975	Res Surg Oncol
UCLA Sch of Medicine, LA	Residency	1972-1980	Surg/Thorac Surg

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1980—86	Senior Investigator, Surgery Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland
1980—86	Lecturer in Surgery, Johns Hopkins Univ Sch of Medicine, Baltimore, Maryland
1982—86	Clinical Assistant Professor, Department of Surgery, School of Medicine, The Medical Center, Georgetown University, Washington, D.C.
1982—86	Head, Thoracic Oncology Section, Surgery Branch,Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
1986—P	Bud S. Johnson Professor of Thoracic Surgery and Chairman, Department of Thoracic Surgery, UT M. D. Anderson Cancer Center, Houston, Texas
1986—P	Professor of Tumor Biology, Department of Tumor Biology, M. D. Anderson Cancer Center, Houston, TX
1987—P	Faculty Appointment, Graduate School of Biomedical Sciences, The University of Texas Medical Sch, Health Science Ctr, Houston, TX
Nov 1991-Aug 1992	Director of the Lung Cancer Research Program, Dept of Thoracic Surgery, in the Division of Surgery, UTMDACC, Houston, Texas

Honors and Awards:

1967	B.A. Magna cum Laude with distinction in all subjects, Phi Beta Kappa, Cornell University, Ithaca, New York
1971	Outstanding Student Research Presentation, Johns Hopkins Medical Society
1975	James Ewing Society Resident—Fellow Clinical Research Award
1977	Society of Surgical Oncology Resident—Fellow Award
1981	Special Achievement Award, U.S. Department of Health and Human Services
1983	Who's Who in Frontier Science and Technology
1987	Recipient of Merit Award, Grant R01 CA45187 from Division of Research Grants and Biologic Response Modifiers Program, National Institutes of Health
1990	Who's Who in Health and Medical Services
1991	Who's Who in Science and Engineering
1991	The Best Doctors in America
1992	Lucy Wortham James Basic Research Award (SSO)

Selected Publications

Yang SC, Owen—Schaub L, Mendiguren—Rodriguez A, Grimm EA, Hong WK, **Roth JA**: Combination immunotherapy for non—small cell lung cancer: Results using interleukin—2 and tumor necrosis factor—α. J Thorac Cardiovasc Surg 99:8-13, 1990.

Yang SC, Owen—Schaub LB, **Roth JA**, Grimm EA: Characterization of OKT3—initiated lymphokine—activated effectors expanded with interleukin—2 and tumor necrosis factor—α. Cancer Res. 50(16):3526-3532, 1990.

Putnam JB, Lammermeier DE, Colon R, McMurtrey MJ, Ali MK, **Roth JA**: Predicted pulmonary function and survival after pneumonectomy for primary lung carcinoma. Ann Thor Surg 49:909-915, 1990.

Schneider PM, Hung M-C, Ames RS, Putnam EA, Akpakip B, **Roth JA**: A novel alteration in the epidermal growth factor receptor gene is frequently detected in human non-small cell lung cancer. Lung Cancer 6:65-72, 1990.

Ellerbroek NA, Fossella FV, Rich TA, Ajani JA, Komaki R, **Roth JA**, Holoye PY: Low-dose continuous infusion cisplatinum combined with external beam irradiation for advanced colorectal adenocarcinoma and unresectable non-small cell lung carcinoma. Inter J Radia Oncol, Biol, Phys 20(2):351-355, 1991.

Mukhopadhyay T, Tainsky M, Cavender AC, **Roth JA**: Specific inhibition of K-ras expression and tumorigenicity of lung cancer cells by antisense RNA. Cancer Res 51:1744-1748, 1991.

Pass HI, Evans S, Matthews WA, Perry R, Venzon D, **Roth JA**, Smith P: Photodynamic therapy of oncogene-transformed cells. J Thorac Cardiovasc Surg 101:795-799, 1991.

Yang SC, Grimm EA, **Roth JA**: Biologic therapy for lung cancer. The Cancer Bulletin 43(2)100-108, 1991.

Lazenby AW, Yang SC, Sniege N, Grimm EA, **Roth JA**: Regression of radiation and chemotherapy resistant non-small cell lung cancer treated with OKT3, interleukin-2, and tumor necrosis factor. The Cancer Bulletin 43(2):181-184, 1991.

Slater JD, Ellerbroek NA, Barkley HT, Mountain C, Oswald MJ, **Roth JA**, Peters LJ: Radiation therapy following resection of non—small cell bronchogenic carcinoma. Intern J Radia Oncol, Biol, Phys. 20:945-951, 1991.

Yang SC, Grimm EA, Parkinson DR, Carinhas J, Fry KD, Rodriguez A, Licciardello J, Owen-Schaub LB, Hong WK, **Roth JA**: Clinical and immunomodulatory effects of combination immunotherapy with low-dose IL-2 and tumor necrosis factor-alpha in patients with advanced non-small cell lung cancer: A phase I trial. Cancer Res 51:3669-3679, 1991.

Casson AG, Mukhopadhyay T, Cleary KR, Ro JY, Levin B, **Roth JA**: p53 gene mutations in premalignant Barrett's epithelium. Cancer Res 51(16):4495-4499, 1991.

Yang SC, Grimm EA, **Roth JA**: Immunotherapy of lung cancer. Chest Surgery Clinics of North America 1(1):191-204, 1991.

Zatopek NK, Holoye PY, Ellerbroek NA, Hong WK, **Roth JA**, Ryan MB, Komaki R, Pang AC, Glisson BS: Resectability of small-cell lung cancer following induction chemotherapy in patients with limited disease (stage II-IIIb). Am J Clin Oncol 14(5):427-432, 1991.

Putnam EA, Yen N, Gallick GE, Steck PA, Fang K, Akpakip B, Gazdar AF, **Roth JA**: Autocrine growth stimulation by transforming growth factor-α in human non-small cell lung cancer. J Surg Oncol 1:49-60, 1992.

Mukhopadhyay T, **Roth JA**: A simple and efficient method for isolation of DNA fragment from agarose gel. J Nucleic Acids Res 19(23):6656, 1992.

Casson AG, Putnam JB, Natarajan G, Johnston DA, Mountain C, McMurtrey M, **Roth HA**: Five year survival after pulmonary metastases for adult soft tissue sarcoma. Cancer 69(1):662- 668, 1992.

This meeting will be open to the public on June 29 from 8:30 a.m. to 10 a.m. Agenda items to be discussed will include a Report from the Director, NINR; an Administrative Report by the Scientific Review Administrator, and consideration of future meeting dates.

In accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), title 5, U.S. Code and section 10(d) of Public Law 92–463, the meeting will be closed to the public on June 29 from 10 a.m. to adjournment on June 30 for the review, discussion, and evaluation of individual grant applications. The applications and the discussions could reveal confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contract Dr. Ernest Marquez 301–594–7865 in advance of the meeting.

Dr. Mary Stephens-Frazier, Scientific Review Administrator, Nursing Science Review Section, National Institute of Nursing Research, National Institutes of Health, Westwood Building, room 740, Bethesda, Maryland 20892, (301) 594–7865, will provide a summary of the meeting, and a roster of committee members upon request.

(Catalog of Federal Domestic Assistance Program No. 93.361, Nursing Research, National Institutes of Health.)

Dated: April 18, 1994.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 94–9778 Filed 4–21–94, 8:45 am]

BILLING CODE 4140–01–M

National Library of Medicine; Meeting of the Literature Selection Technical Review Committee

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Literature Selection Technical Review Committee, National Library of Medicine, on June 2–3, 1994, convening at 9 a.m. on June 2 and at 8:30 a.m. on June 3 in the Board Room of the National Library of Medicine, Building 38, 8600 Rockville Pike, Bethesda, Maryland.

The meeting on June 2 will be open to the public from 9 a.m. to approximately 11 a.m. for the discussion of administrative reports and program developments. Attendance by the public will be limited to space available. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Ms. Karen Griffin at 301–496–6921 two weeks before the meeting.

In accordance with provisions set forth in section 552b(c)(9)(B), Title 5, U.S.C., Public Law 92–463, the meeting will be closed on June 2 from 11 a.m. to approximately 5 p.m. and on June 3 from 8:30 a.m. to adjournment for the review and discussion of individual journals as potential titles to be indexed by the National Library of Medicine. The presence of individuals associated with these publications could hinder fair and open discussion and evaluation of individual journals by the Committee members.

Mrs. Lois Ann Colaianni, Scientific Review Administrator of the Committee, and Associate Director, Library Operations, National Library of Medicine, 8600 Rockville Pike, Bethesda, Maryland 20894, telephone number: 301–496–5921, will provide a summary of the meeting, rosters of the committee members, and other information pertaining to the meeting.

Dated: April 18, 1994.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 94–9775 Filed 4–21–94; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on June 9–10, 1994. The meeting will be held at the National Institutes of Health, Building 31C, 6th Floor, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on June 9, 1994, to adjournment at approximately 5 p.m. on June 10, 1994. The meeting will be open to the public to discuss Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958) and other matters to be considered by the Committee. The Proposed Actions to be discussed will follow this notice of meeting.

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496–9838, FAX (301) 496–9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: April 18, 1994.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 94–9789 Filed 4–21–94; 8:45 am]

BILLING CODE 4140–01–M

National Institutes of Health (NIH); Meeting of Panel/Request for Public Comment

The fourth meeting of the National Institutes of Health (NIH) Human Embryo Research Panel will be held May 3–4 from 9 a.m. to 5 p.m. each day at the Holiday Inn Bethesda, 8120 Wisconsin Avenue, Bethesda, Maryland. The Panel is a group of special consultants to the Advisory Committee to the Director (ACD), NIH, established to recommend guidelines for Federal funding of research involving the *ex utero* human embryo resulting from *in vitro* fertilization or other sources

Federal Register

Wednesday
May 11, 1994

Part IX

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Proposed Actions Under the Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of proposed actions under the NIH guidelines for research involving recombinant DNA molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee at its meeting on June 9–10, 1994. After consideration of these proposals and comments by the Recombinant DNA Advisory Committee, the Director of the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by May 26, 1994, will be reproduced and distributed to the Recombinant DNA Advisory Committee for consideration at its June 9–10, 1994, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301–496–9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules:

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Sobol and Royston

In a letter dated October 6, 1993, Drs. Robert Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, submitted the human gene transfer protocol entitled: Injection of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL–2): A Phase I Study to the Recombinant DNA Advisory Committee for formal review and approval. At the December 2–3, 1993, meeting, the Recombinant DNA Advisory Committee disapproved the original protocol. The majority of the Recombinant DNA Advisory Committee members concluded that the preclinical data derived from a previous single patient protocol was inadequate to justify the experiment. The motion to disapprove the protocol passed by a vote of 10 in favor, 5 opposed, and 1 abstention.

In a letter dated April 8, 1994, Drs. Sobol and Royston submitted a revised protocol to the Recombinant DNA Advisory Committee for formal review and approval.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Curiel

In a letter dated April 13, 1994, Dr. David Curiel of the University of Alabama, Birmingham, Alabama, submitted the human gene transfer protocol entitled: Phase I Trial of a Polynucleotide Vaccine to Human Carcinoembryonic Antigen in Patients with Metastatic Colorectal Cancer to the Recombinant DNA Advisory Committee for formal review and approval.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Evans and Robbins

In a letter dated April 13, 1994, Drs. C. H. Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, submitted the human gene transfer protocol entitled: Clinical Trial to Assess the Safety, Feasibility, and Efficacy of Transferring a Potentially Anti-arthritic Cytokine Gene to Human Joints with Rheumatoid Arthritis to the Recombinant DNA Advisory Committee for formal review and approval.

IV. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Heslop, Brenner, and Krance

In a letter dated April 6, 1994, Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of the St. Jude Children's Research Hospital, Memphis, Tennessee, submitted the human gene transfer protocol entitled: Use of Double Marking with Retroviral Vectors to Determine Rate of Reconstitution of Untreated and Cytokine Expanded CD34(+) Selected Marrow Cells in Patients Undergoing Autologous Bone Marrow Transplantation to the Recombinant DNA Advisory Committee for formal review and approval.

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Lyerly

In a letter dated April 12, 1994, Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, submitted the human gene transfer protocol entitled: A Pilot Study of Autologous Human Interleukin-2 Gene Modified Tumor Cells in Patients with Refractory or Recurrent Metastatic Breast Cancer to the Recombinant DNA Advisory Committee for formal review and approval.

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Economou, Glaspy, and McBride

In a letter dated April 11, 1994, Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California, submitted a human gene transfer protocol entitled: A Phase I Testing of Genetically Engineered Interleukin-7 Melanoma Vaccines to the Recombinant DNA Advisory Committee for formal review and approval.

VII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Freedman

In a letter dated March 22, 1993, Dr. Ralph Freedman of M.D. Anderson Cancer Center, Houston, Texas, submitted the human gene transfer protocol entitled: Use of a Retroviral Vector to Study the Trafficking Patterns of Purified Ovarian Tumor Infiltrating Lymphocyte (TIL) Populations Used in Intraperitoneal Adoptive Immunotherapy of Ovarian Cancer Patients: A Pilot Study to the Recombinant DNA Advisory Committee for formal review and approval. At its June 7–8, 1993, meeting the Recombinant DNA Advisory Committee deferred the protocol until the investigators return to the full Recombinant DNA Advisory Committee with the following:

(1) Data demonstrating efficient transduction of TIL,

(2) Sufficient information regarding demonstration of selectivity, i.e., specific trafficking of TIL to tumor,

(3) Complete statistical analysis,

(4) Revised Informed Consent document in simplified language, and

(5) Address concerns about patient responsibility for research-related costs.

The motion to defer the protocol

pending full Recombinant DNA Advisory Committee review of additional information passed by a vote of 18 in favor, 0 opposed, and no abstentions.

In a letter dated January 5, 1994, Dr. Freedman submitted a revised protocol to the Recombinant DNA Advisory Committee for formal review and approval. At its March 3–4, 1994, meeting, the Recombinant DNA Advisory Committee deferred the protocol until the investigator returns to the full Recombinant DNA Advisory Committee with:

(1) A modified protocol, which includes a revised treatment schema that will provide statistically significant information, and

(2) A revised Informed Consent document that adequately describes the procedures that will be performed in language understandable to lay persons. The motion to defer the protocol pending full Recombinant DNA Advisory Committee review of the additional information passed by a vote of 12 in favor, 1 opposed, and no abstentions.

In a letter dated April 13, 1994, Dr. Freedman submitted a revised protocol to the Recombinant DNA Advisory Committee for formal review and approval.

VIII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Deisseroth, Hortobagyi, and Champlin

In a letter dated April 12, 1994, Drs. Albert Deisseroth, Gabriel Hortobagyi, and Richard Champlin of the M.D. Anderson Cancer Center, Houston, Texas, submitted the human gene transfer protocol entitled: Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Breast Cancer: A Pilot Trial to the Recombinant DNA Advisory Committee for formal review and approval.

IX. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Roth

In a letter dated April 12, 1994, Dr. Jack Roth of M.D. Anderson Cancer Center, Houston, Texas, submitted the human gene transfer protocol entitled: Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin to the Recombinant DNA Advisory Committee for formal review and approval.

X. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Lotze

In a letter dated April 13, 1994, Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania submitted the human gene transfer protocol entitled: IL–12 Gene Therapy Using Direct Injection of Tumor with Genetically Engineered Autologous Fibroblasts to the Recombinant DNA Advisory Committee for formal review and approval.

XI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Liu and Young

In a letter dated October 7, 1993, Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, submitted the human gene transfer protocol entitled: Retroviral Mediated Gene Transfer of the Fanconi Anemia Complementation Group C Gene to Hematopoietic Progenitors of Group C Patients to the Recombinant DNA Advisory Committee for formal review and approval. At the December 2–3, 1993, meeting, the Recombinant DNA Advisory Committee deferred the protocol until the investigators return to the full Recombinant DNA Advisory Committee with the following:

(1) Murine data demonstrating *in vivo* expression of the FACC gene and safety data accumulated over a period of ≥ 4 months demonstrating that the FACC-transduced cells do not produce any untoward effects, i.e., malignant transformation:

(2) Data (cited in Dr. Cynthia Dunbar's December 1993 data management report, Protocol #9206–025) regarding the possibility that "stem cell factor could favor the growth of leukemic versus normal progenitors during *ex vivo* culture periods;" and

(3) Revised eligibility criteria sections for both the protocol and Informed Consent document that describe the necessity for bone marrow examination following each infusion.

The consensus of the Recombinant DNA Advisory Committee was that the investigators were not required to submit this additional data until 4 weeks prior to the Recombinant DNA Advisory Committee meeting at which the information is reviewed. Submission of previously reviewed information is not required. The motion to defer the protocol pending full Recombinant DNA Advisory Committee review of additional information passed by a vote of 14 in favor, 0 opposed, and 3 abstentions.

On May 2, 1994, Drs. Liu and Young submitted additional materials relating to the human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval.

XII. Amendment to Part I–D of the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects, NIH Guidelines, Regarding Informed Consent/Dr. Zallen

During the December 2–3, 1993, Recombinant DNA Advisory Committee meeting, Dr. Gary Ellis, Director of the Office for Protection from Research Risks (OPRR), NIH, Bethesda, Maryland, responded to the written comments submitted by Dr. Zallen, Chair of the Working Group on Informed Consent Issues. Dr. Ellis noted the Recombinant DNA Advisory Committee's concern regarding specific issues that should be addressed in human gene transfer protocol Informed Consent documents, i.e., request for autopsy, recommendations for male/female contraception, separate Informed Consent documents when gene therapy is separate from a clinical protocol, commitment to long-term patient follow-up, and financial responsibility of the institution for all research-related costs. During his presentation, Dr. Ellis provided the Recombinant DNA Advisory Committee with background information regarding the roles of both OPRR and local Institutional Review Boards (IRB) in the review of research proposals involving human subjects. Dr. Ellis recommended that the Recombinant DNA Advisory Committee draft a letter outlining its specific recommendations to OPRR for distribution and consideration by the local IRBs.

In a memorandum dated December 23, 1993, Dr. Ellis further clarified the avenues that should be pursued by the Recombinant DNA Advisory Committee with regard to the "quality and content of informed consent documents into constructive changes in the informed consent process," specifically in relation to human gene transfer. Dr. Ellis recommended that the Points to Consider should be amended to introduce consistency in the Informed Consent document language.

During the March 3–4, 1994, Recombinant DNA Advisory Committee meeting, Dr. Doris Zallen, Chair of the Working Group on Informed Consent, provided a summary of the proposed amendments to Part I–D, Informed Consent of the Points to Consider. Two versions of revised Part I–D were presented:

(1) The version drafted by the working group, and

(2) A modified version incorporating the modifications suggested by Mr. Alex Capron. The Recombinant DNA Advisory Committee recommended that the working group should develop a consolidated version of part I–D which includes language from both proposed documents. The Recombinant DNA Advisory Committee suggested that questions should be prefaced with an explanation as to the necessity for the requested information.

On April 27, 1994, Dr. Zallen submitted revised amendments to part I–D, Informed Consent, of the Points to Consider in response to the specific comments posed by the Recombinant DNA Advisory Committee at its March 3–4, 1994, meeting. The proposed amendments read:

Part I–D Informed Consent

"In accordance with the requirements of DHHS regulations for the protection of human subjects (45 CFR part 46), investigators shall indicate how subjects will be informed about the proposed study, the manner in which their consent will be solicited, and that the informed consent form makes clear the special requirements of gene transfer research.

Part 1–D–1. Communication of the Study to Potential Participants

Part I–D–1–a. Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid potential conflicts of interest if the investigator is also providing medical care to potential subjects?

Part I–D–1–b. Where will discussions or other means of informing individuals about the proposed study take place?

Part I–D–1–c. How will the major points covered in Parts I–A through I–C of the Points to Consider be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

Part I–D–1–d. What is the length of time that the potential participants will have to make a decision about their participation in the study?

Part I–D–1–e. If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

Part I–D–2. Informed Consent Document

"Investigators submitting human gene transfer proposals for Recombinant DNA Advisory Committee review must include the Informed Consent document as approved by the local Institutional Review Board. A separate consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, such as when the gene is used as a 'marker' or when it is used to enhance the power of immunotherapy for cancer.

"Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the possibility that many of the potential risks remain undefined, the Informed Consent document shall include the following specific information in addition to any requirements of the DHHS regulations for the protection of human subjects (45 CFR part 46). Indicate if each of the specified items appears in the consent form or, if not in the consent form, how those items will be presented to potential subjects. Include an explanation if any of the following items is omitted from the consent process or document.

Part I–D–2–a. General Requirements of Human Subjects Research

Part I–D–2–a–(1). Description/purpose of study. "The subjects should be provided a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene-transfer component.

Part I–D–2–a–(2). Alternatives. "The consent form should indicate the availability of other therapies, including the possibility of other investigational therapies and approaches.

Part I–D–2–a–(3). Voluntary participation. "The subjects should be informed that participation in the study is voluntary and that failure to participate in the study, or withdrawal of consent, will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

Part I–D–2–a–(4). Benefits. "The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For experiments which are not reasonably expected to provide a therapeutic benefit to subjects, the consent form shall clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

Part I–D–2–a–(5). Possible risks, discomforts, and side effects. "There should be a clear itemization in the consent form of types of adverse experiences, the relative severities, and the expected frequencies. For consistency of definition, side effects that are listed as mild should be ones which do not require a therapeutic intervention. Moderate side effects require an intervention. Severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization. Rare side effects occur in less than one in one thousand subjects, uncommon side effects in less than 1% of subjects, common side effects in one to 10% of subjects, and frequent side effects are those which occur in more than 10% of subjects.

"The consent form should provide information regarding the approximate number of people who have received the genetic material under study. It is also necessary to warn potential subjects that for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

"Any possible adverse medical consequences that may occur if the subjects withdraw from the study once the experiment has started should be indicated.

"Part I–D–2–a–(6). Costs. "The subjects should be provided with information about any financial costs associated with their participation in the experiment and in the long-term follow-up to the experiment that are not covered by the investigators or the institutions involved. Comparable financial information for other available alternatives, including other investigational therapies, should also be provided.

"In the consent form, subjects should be informed about the extent to which they will be responsible for any costs for medical treatment required as a direct result of research-related injury.

Part I–D–2–b. Specific Requirements of Gene Transfer Research

Part I–D–2–b–(1). Use of barrier contraception. "To avoid the possibility that any of the reagents employed in gene transfer research could cause harm to a developing fetus, female subjects should be informed that they should not be pregnant during the course of their participation in the study. Both male and female subjects should be informed when barrier contraception is required during the active phase of their participation in the study.

Part I–D–2–b–(2). Long-term follow up. "To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. A list should be provided in the consent

form of persons who can be contacted in the event that questions arise during the follow-up period. The principal investigator should request that subjects always keep the laboratory informed of a current address and telephone number.

"The subjects should also be informed that any significant findings resulting from the study will be made known to them and/or their parent or guardian including new information about the experimental procedure, the physical reactions experienced by other individuals involved in the study, and any long-term effects that have been noted.

"Part I–D–2–b–(3). Request for autopsy. "To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, whenever that may occur, an autopsy will be requested and that they should advise their families of this request and of its scientific and medical importance.

Part I–D–2–b–(4). Interest of media and others in the research. To alert the subjects that others may have an interest in the innovative character of the experiment and the status of treated subjects:

"The subjects should be informed that the institution and investigators will make every effort to provide protection from the media in an effort to protect participants' privacy;

"The subjects should be informed that representatives of applicable Federal agencies (e.g., NIH, Food and Drug Administration), representatives of collaborating institutions, vector suppliers, etc., will have access to medical records of the participants."

XIII. Deletion of Appendix L of the NIH Guidelines Regarding Release Into the Environment/Dr. Wivel

On April 29, 1994, Dr. Nelson Wivel of the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested that Appendix L, Release into the Environment of Certain Plants, be deleted from the NIH Guidelines.

The Office of Recombinant DNA Activities (ORDA) requests that Appendix L, Release into the Environment of Certain Plants, be deleted from the NIH Guidelines based on the following:

(1) Section I of the NIH Guidelines allows experiments to proceed that are reviewed and approved by another Federal agency that has jurisdiction for review and approval without the necessity for NIH review or approval (52 FR 31849);

(2) The Recombinant DNA Advisory Committee has not reviewed any deliberate release experiment involving recombinant DNA since 1984;

(3) At its May 30–31, 1991, meeting, the Recombinant DNA Advisory Committee recommended that Section III–A–2 be deleted from the NIH Guidelines; and

(4) Experiments involving deliberate release into the environment are currently reviewed within the framework of existing Federal regulations, i.e., the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA).

Section I of the NIH Guidelines was amended on August 24, 1987, such that any recombinant DNA experiment (other than human gene transfer) may proceed without Recombinant DNA Advisory Committee and NIH approval if it has been reviewed and approved by another Federal agency that has jurisdiction over such a proposal. The amended version (52 FR 31849) of Section I reads as follows:

Section I–A. Purpose

"* * * Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, has been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval * * *."

On December 6, 1990, the Recombinant DNA Advisory Committee Planning Subcommittee recommended that the requirement for Recombinant DNA Advisory Committee review of experiments involving deliberate environmental release of organisms containing recombinant DNA be eliminated from the NIH Guidelines. This recommendation reflected the fact that the Federal regulatory agencies, the USDA and EPA, are responsible for the review and approval of environmental release experiments. The Recombinant DNA Advisory Committee reviewed the request and recommended that the following sections be deleted from the NIH Guidelines:

Section III–A–2

Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term "deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section III–A–2–a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in appendix L.

Section III–A–2–b. Deletion derivatives and single base changes not otherwise covered by the NIH Guidelines.

Section III–A–2–c. For extrachromosomal elements and microorganisms (including viruses), rearrangements and amplifications within a single genome. Rearrangements involving the introduction of DNA from different strains of the same species would not be covered by this exemption.

Based on these amendments to the NIH Guidelines, that have previously been recommended by the Recombinant DNA Advisory Committee, and the fact that the principals of planned introduction are now in place which provide a risk-assessment method by other Federal regulatory agencies, the Office of Recombinant DNA Activities requests that Appendix L be deleted from the NIH Guidelines.

Appendix L will be deleted as follows:

Appendix L. Release Into the Environment of Certain Plants

Appendix L–I. General Information

"Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Working Group, and specific approval by the NIH Director. Such experiments also require the approval of the IBC before initiation. Information on specific experiments which have been approved will be available in ORDA and will be listed in appendix L–III when the Guidelines are republished.

"Experiments which do not meet the specifications of appendix L–II fall under section III–A and require RAC review and NIH and IBC approval before initiation.

Appendix L–II. Criteria Allowing Review by the RAC Plant Working Group Without the Requirement for Full RAC Review

"Approval may be granted by ORDA in consultation with the Plant Working Group without the requirement for full RAC review (IBC review is also necessary) for growing plants containing recombinant DNA in the field under the following conditions:

Appendix L–II–A. The plant species is a cultivated crop of a genus that has no species known to be a noxious weed.

Appendix L–II–B. The introduced DNA consists of well/characterized genes containing no sequences harmful to humans, animals, or plants.

Appendix L–II–C. The vector consists of DNA:

(i) From exempt host-vector systems (see Appendix C);

(ii) From plants of the same or closely related species;

(iii) From nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants;

(iv) From plant pathogens only if sequences resulting in production of disease symptoms have been deleted; or

(v) Chimeric vectors constructed from sequences defined in (i) or (iv) above. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests.

Appendix L–II–D. Plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review by the IBC. Copies must also be submitted to the Plant Working Group of the RAC.

XIV. Amendment to Part VI of the Points To Consider, NIH Guidelines, Regarding Expedite Review/Dr. Wivel

On April 29, 1994, Dr. Nelson Wivel of the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested that part VI, Procedures to be Followed for Expedited Review, of the Points to Consider be amended to clarify submission requirements for Expedited Review.

The Procedures to be Followed for Expedited Review currently reads:

"4. Regardless of the method of review, the Points to Consider must be the standard review for all gene transfer protocols."

The proposed amendment reads:

"4. Regardless of the method of review, the Points to Consider must be the standard review for all gene transfer protocols; therefore, submission of the Points to Consider is required."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: May 4, 1994.

Suzanne Medgyesi-Mitschang,

Acting Deputy Director for Science Policy and Technology Transfer.

[FR Doc. 94–11466 Filed 5–10–94; 8:45 am]

BILLING CODE 4140–01–P

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 9-10, 1994

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
June 9-10, 1994

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-eighth meeting at 9:00 a.m. on June 9, 1994, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Nancy L. Buc, Buc, Levitt, & Beardsley
Gary A. Chase, Georgetown University Medical Center
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, The Foundation of Human Genetics
David Ginsburg, University of Michigan
Robert Haselkorn, University of Chicago
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington
Robertson Parkman, Children's Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Gail S. Ross, Cornell University Medical Center
Bratin K. Saha, Emory University
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, Virginia Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).

Ad Hoc Consultant

Harold Ginsberg, National Institutes of Health/Columbia University

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[1]The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

Niles Bernick, OD
R. Michael Blaese, NCI
Diane Bronzert, NCI
Sarah Carr, OD
Barry Goldspiel, CC
Toby Hecht, NCI
Christine Ireland, OD
Susan Jenks, NCI
Becky Ann Lawson, OD
Johnson Liu, NHLBI
Catherine McKeon, NIDDK
Koichi Miyamura, NHLBI
Joan Porter, OD
Muzaffar Quazilbash, NHLBI
Mallika Sekhar, NHLBI
Thomas Shih, OD
Bernard Talbot, NCRR
Chris Walsh, NHLBI
Debra Wilson, OD
Neal Young, NHLBI

Others:

Munir Abdullah, Glaxo, Inc.
Estuardo Aguilar, Baylor College of Medicine
W. French Anderson, University of Southern California
Mary Helen Binger, Hoffmann-LaRoche, Inc.
Bridget Binko, Cell Genesys, Inc.
Robert Boyd, Knight-Ridder, Inc.
Ronna Campbell, University of Pittsburgh
Jeff Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
Barry Carter, Targeted Genetics Corporation
Jan Chappell, Genetic Therapy, Inc.
Yawen Chiang, Genetic Therapy, Inc.
Lori Clarke, Genetic Therapy, Inc.
Richard Cote, University of Southern California
David Curiel, University of Alabama
Karen Darcy, Magenta Corporation
Albert Deisseroth, MD Anderson Cancer Center

James Economou, University of California, Los Angeles
Chris Evans, University of Pittsburgh
Chuck Finn, Genetics Institute, Inc.
Olivera Finn, University of Pittsburgh
Terence Flotte, Johns Hopkins Hospital
Suzanne Forry-Schaudies, Genetic Therapy, Inc.
Ralph Freedman, MD Anderson Cancer Center
Joyce Frey, Food and Drug Adminstration
Lynn Frohnmayer, Mothers of Children with Fanconi Anemia
Morgan Gale, Hearings-on-the-line
Diane Gershon, Nature Magazine
Richard Giles, MD Anderson Cancer Center
Alan Goldhammer, Biotechnology Industry Organization
Richard Gregory, Canji, Inc.
Robert Grossman, Arrow International Marketing, Inc.
Elie Hanania, MD Anderson Cancer Center
Helen Heslop, St. Jude Childrens Research Hospital
Frankie Holmes, MD Anderson Cancer Center
David Holzman, BioWorld
Joseph Hughes, Quality Biotech
Edie Irvine, Genetic Therapy, Inc.
Jeffrey Isner, St. Elizabeths Medical Center
Bobbi Kamil, Cable in the Classroom
Michael Kanaley, The Novus Group
Karilyn Kelson, Mothers of Children with Fanconi Anemia
Martin Korbling, MD Anderson Cancer Center
Toshi Kotani, Genetic Therapy, Inc.
Steven Kradjian, Vical, Inc.
Gary Kurtzman, Avigen, Inc.
Al Kuta, Food and Drug Administration
Albert Lobuglio, University of Alabama
Michael Lotze, University of Pittsburgh
Bill Macaulay, University of Pittsburgh
Tamie Malaska, Targeted Genetics Corporation
Dan Maneval, Canji, Inc.
Tony Marcel, TMC Development
Eliot Marshall, Science Magazine
John Marshall, Magenta Corporation
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Andra Miller, Food and Drug Administration
Grant Mitchell, St. Justin Hospital
Micheal Nash, MD Anderson Cancer Center
Philip Noguchi, Food and Drug Administration
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Magenta Corporation

Robert Overell, Targeted Genetics Corporation
Andrea Pavirani, Transgene, Inc.
Michael Penn, Genetic Therapy, Inc.
Michael Pensiero, Genetic Therapy, Inc.
Jerri Perkins, Perkins & Perkins
Joan Personett, American College of Obstetricians and Gynecologists
Stephen Pijar, University of Maryland
Chris Platsoucas, Temple University School of Medicine
John Powderly, Georgetown Medical School
Raj Puri, Food and Drug Administration
Kathryn Pushkar, The Blue Sheet
Urban Ramstedt, Virus Research Institute
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
Paul Robbins, University of Pittsburgh
Jack Roth, MD Anderson Cancer Center
Leonard Schiff, Microbiological Associates, Inc.
Richard Scotland, Genzyme Corporation
Tomiko Shimada, Ambience Awareness International
John Shiver, Merck, Inc.
Robert Sobol, San Diego Regional Cancer Center
Ruth SoRelle, Houston Chronicle
Margi Stuart, Breast Cancer Action
Frank Sturtz, Progenitor, Inc.
Hideaki Tahara, University of Pittsburgh
Tom Tarlow, Chiron Corporation
Paul Tolstoshev, Genetic Therapy, Inc.
Matthew Tomaino, University of Pittsburgh
Karl Uhlendorf, The Blue Sheet
Cynthia Utley, GenVec
Dominick Vacante, Microbiological Associates, Inc.
Deborah Vaz, Virus Research Institute
Wanda de Vlaminck, Avigen, Inc.
Kenneth Walsh, St. Elizabeth Medical Center
Judi Weissinger, Applied Immune Sciences
Katharine Whartenby, Food and Drug Adminstration
Sharon Williams, Life Technologies, Inc.
Wei-Wei Zhang, MD Anderson Cancer Center
Laurence Zitvogel, University of Pittsburgh

I. CALL TO ORDER/DR. WALTERS

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting was published in the *Federal Register* on April 22, 1994 (59 FR 19200), and the proposed actions were published in the *Federal Register* on May 11, 1994 (59 FR 24618), as required by the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the proposal, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, and followed by the public at large.

New Members

Dr. Walters said that three new RAC members will participate in this meeting: (1) Gail S. Ross, Ph.D., Research Director, High-risk Infant Follow-up Program, New York Hospital, Perinatology Center, Cornell University, New York, New York; (2) Bratin K. Saha, Ph.D., Assistant Professor, Department of Pathology, Winship Cancer Center, Emory University, Atlanta, Georgia; and (3) David Ginsburg, M.D., Professor, Department of Internal Medicine and Human Genetics, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan.

Overview

Dr. Walters noted that 10 human gene transfer protocols will be reviewed at this meeting, 8 gene therapy protocols (6 cancer, 1 rheumatoid arthritis, and 1 Fanconi anemia) and 2 gene marking studies (1 autologous bone marrow marking and 1 tumor infiltrating lymphocyte marking).

Discussion--Compensation for Research-Related Injuries

Dr. Walters summarized the meeting material regarding compensation for research-related injuries that was provided as a supplement to the RAC's previous discussions regarding provision of medical care to subjects who may be injured during the course of their participation in research.

Robert Levine's book entitled, *Ethics and Regulation of Clinical Research*, includes a discussion of the Yale University program on compensation for research-related injuries.

The University of Washington has a self-insured Human Subjects Compensation plan for research protocols that are not beneficial or therapeutic. Since 1979, the University of Washington plan has received 21 requests for payment resulting in a total outlay of $4,110.70. The most costly incident involved an elderly subject who broke her leg while participating in a fall prevention study. The injured subject required treatment costing $1,639.04. Most other requests averaged less than $600.

The Council for International Organization of Medical Sciences, Geneva, Switzerland, has published the *International Ethical Guidelines for Biomedical Research Involving Human Subjects*. These guidelines specify therapies that are provided free of charge for specific research-related injuries, and asserts the right of subjects to compensation in the event of disability or death.

On February 24, 1990, the U.S. Department of Defense (Army) issued regulations on the *Use of Volunteers as Subjects of Research*. According to these regulations, subjects who receive research-related injuries while in uniform receive compensation comparable to subjects who have been injured in combat. Civilian employees are covered under Worker's Compensation. Medical insurance and direct costs for contracting personnel are provided as part of the contract cost negotiated between the U.S. Department of Defense (Army) and the contractor.

Documents are included from the Royal College of Physicians and the Association of the British Pharmaceutical Industry that detail Great Britain's National Health System in which all medical costs are covered for subjects injured during the course of their participation in research.

Dr. Zallen inquired about the current status of legislation relating to U.S. Health Care Reform and coverage for research-related injuries. Dr. Wivel explained that there are several health care proposals pending consideration at different Congressional committees and that specific language regarding compensation for research-related injuries has not been finalized in the legislation.

Temin Letter Regarding Potential for Recombination of Retroviral Vectors

Dr. Walters noted a letter dated December 2, 1993, from Dr. Howard M. Temin requesting that the RAC consider a specific safety issue regarding the potential for recombination of retroviral vectors. The letter states:

> "I would like you to consider the following safety point relative to retrovirus vectors. Recombination, which could form replication-competent virus by recombination between the vector and packaging cell RNAs, only takes place upon infection of sensitive cells. Thus, even if there is no replication-competent virus in a vector inoculum, replication-competent virus could be formed upon infection. Does the testing now used for vector preparations used in patients adequately test for this possibility?"

Included in the meeting materials were comments by RAC members and representatives of industry responding to Dr. Temin's letter. Since this item was not included in the meeting agenda announced in the *Federal Register*, the discussion remained informal. Dr. Walters suggested that a working group should be established prior to the next RAC meeting to determine the RAC's plan of action with regard to this issue and invited suggestions for *ad hoc* consultants who could provide their expert comments on this issue.

Dr. Miller said that this issue is basically academic since current replication-competent retrovirus (RCR) assays adequately test for the recombination events alluded to by Dr. Temin. The standard S^+L^- marker rescue assays and the *Mus dunni* co-cultivation assay are sensitive enough to detect RCR both in the vector preparations and viruses generated following cell infection. Dr. Parkman concurred with Dr. Miller's statement. Dr. Post said that the question raised by Dr. Temin was not stated explicitly; and unfortunately, the question cannot be clarified since Dr. Temin is recently deceased. Dr. Post speculated that if the helper virus RNA is co-packaged with the vector RNA in virus particles, recombination might occur between these two RNA species upon infection of target cells to generate a novel retrovirus. Dr. Miller said that the current packaging cell lines are constructed with a split helper virus genome in order to reduce the probability of such a recombination event. Dr. Straus said that the question raised by a prominent scientist and Nobel laureate such as Dr. Temin could possibly be more profound that it initially appears. Perhaps, Dr. Temin was speculating about the possibility of generating RCR upon infection of sensitive cells *in vivo*, i.e., in the patient's body, as a result of recombination between the vector sequences and some endogenous elements within the target cells. Dr. Straus said that this issue should be carefully considered by the RAC to assure that current safeguards and testing procedures are adequate. Dr. Miller said that the current RCR testing procedures are designed to screen for such recombination events both before and after infection with the vector preparations. Dr. Philip Noguchi, Director of the Division of Cellular and Gene Therapies, Food and Drug Administration (FDA), agreed with Dr. Miller's statement that current testing procedures can adequately detect most occurrences of RCR; however, remote recombination events do occur during large-scale vector production. Dr. Noguchi encouraged the RAC to continue its discussion of this safety issue.

Update on *Accelerated Review* Procedures

Dr. Walters inquired about the status of the *Accelerated Review* procedures approved by the RAC at its March 1994 meeting. Dr. Wivel responded that these actions are included in the new incorporated version of the *NIH Guidelines*, which is in the final stage of approval by the NIH Director. The 1994 *NIH Guidelines* include new Appendices P and Q that address physical and biological containment for transgenic plants and animals in the greenhouse and animal facility settings. The environmental assessment for these new appendices has been recently approved.

Continuation of Discussion on Compensation for Research-Related Injuries

In regard to the RAC's earlier discussion regarding the provision of medical care to subjects injured during the course of their participation in research, Dr. Walters asked Dr. Smith to comment on Yale University's program for compensation for research-related injuries. Dr. Smith responded that Yale University provides coverage for all costs related to research-related injuries. Although he did not have data regarding the number and amounts of payments for claims under the program, he understands that the claims have not been overwhelming.

II. CHAIR REPORT ON MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that minor modifications were approved to the following human gene transfer protocols since the March 3-4, 1994, RAC meeting (Attachment II):

3/23/94	Protocol #9309-053	Investigators: Peter Cassileth/Eckhard Podack
3/23/94	Protocol #9206-023	Investigator: Cynthia Dunbar
3/23/94	Protocol #9206-025	Investigator: Cynthia Dunbar
4/11/94	Protocol #9212-034	Investigator: Ronald Crystal
4/13/94	Protocol #9303-041	Investigators: Robert Wilmott/Jeffrey Whitsett/Bruce Trapnell
6/01/94	Protocol #9209-020	Investigator: Robert Walker

III. MARCH 3-4, 1994, RAC MINUTES

The RAC approved a motion made by Dr. Chase and seconded by Dr. Doi to accept the March 3-4, 1994, RAC minutes with the inclusion of minor changes suggested by Dr. Chase by a vote of 15 in favor, 0 opposed, and no abstentions.

IV-A. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I TRIAL OF A POLYNUCLEOTIDE AUGMENTED ANTITUMOR IMMUNIZATION TO HUMAN CARCINOEMBRYONIC ANTIGEN IN PATIENTS WITH METASTATIC COLORECTAL CANCER*/DR. CURIEL

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. David Curiel of the University of Alabama, Birmingham, Alabama. Dr. Haselkorn explained that the objective of this Phase I study is to determine whether intramuscular injection of plasmid DNA containing the cDNA for carcinoembryonic antigen (CEA) induces an immune response (humoral or cellular) to CEA in patients with advanced colon carcinoma. CEA is an antigen that is present on the surface of embryonic cells in normal individuals. The immune response that recognizes CEA as a foreign antigen is deleted in early development. However, CEA frequently reappears in certain tumors such as colon carcinoma. The rationale of this study is to immunize cancer patients with the CEA antigen to overcome the immune tolerance and to induce immune response against cancer cells expressing the CEA antigen.

The materials submitted by Dr. Curiel reference an ongoing study conducted by Dr. Jeffrey Schlom of the National Cancer Institute (NCI), NIH. Dr. Schlom's study involves the administration of a recombinant vaccinia viral vector encoding CEA in order to elicit a CEA-specific immune response in cancer patients. In contrast, Dr. Curiel's study involves the administration of a plasmid DNA vector encoding CEA. Dr. Haselkorn

asked the following: (1) How is an anti-CEA immune response expected if there is immune tolerance to the same antigen? (2) Since CEA has a molecular weight of 180,000 daltons and more than half of the mass is attributed to carbohydrate, are enzymes required to add the carbohydrate moiety to the CEA polypeptide? (3) Does antigen/antibody recognition require proper glycosylation of the CEA polypeptide? Dr. Haselkorn said that the original submission was poorly written; however, the investigators have clarified previous concerns in their written responses.

Review--Dr. Chase

Dr. Chase said that the protocol involves intramuscular injection of a plasmid DNA encoding the CEA gene, and the proposed treatment does not carry the risks associated with retrovirus vectors. He objected to the use of the term "vaccine." The term "vaccine" has traditionally been used to describe immunization for the prevention of disease, not treatment of preexisting disease. He objected to the Informed Consent document statement that patients will be responsible for any costs of medical treatment required as a result of research-related injury. Subjects should be informed that no benefit is expected from participation in this study. The Informed Consent document should be revised to reflect these concerns.

Review--Dr. Zallen

Dr. Zallen stated that the investigators have provided complete responses to Section I-D, Informed Consent, of the *Points to Consider*. She noted concern about the statement regarding payment for research-related injuries that medical treatment "is not provided free of charge." The use of passive voice in this statement makes it unclear who will be billed. Will the financial responsibility imposed by this statement constitute a barrier to the participation in this study? She inquired whether animal studies have been conducted to determine if the plasmid DNA spreads beyond the original site of injection.

Other Comments

Dr. Walters noted written comments submitted by Ms. Meyers stating her objection to the use of the term "vaccine" throughout the Informed Consent document and the protocol. Ms. Meyers expressed concern regarding patients' responsibility for costs of medical tests and research-related injuries. Subjects should not be excluded from participation in this study based on their inability to pay for such costs.

Ms. Buc commented that the statement in the Informed Consent document regarding compensation for research-related injuries does not exclude the patient's right to bring a lawsuit if such injuries resulted from negligence. Subjects have the right to choose to enter the protocol. Disclosure is the heart of Informed Consent; therefore, if the terms are clearly disclosed and agreed upon by the subject, the Informed Consent document is considered acceptable. Ms. Buc noted the inappropriateness of the RAC's continued discussions on these Informed Consent issues.

Dr. Smith asked whether there is the potential for toxicity resulting from high levels of CEA and anti-CEA antibodies in the blood. Have these toxicity issues been adequately addressed in animal experiments?

Dr. Parkman expressed concern about the immune response to CEA and immunoprotection of syngeneic CEA expressing tumors in the murine model. The RAC has always required that one consistent criterion for approving any human gene transfer protocol is to demonstrate immunoprotection against a preexisting tumor in an animal model. The preclinical studies submitted by the investigator has not adequately demonstrated immunoprotection. The antitumor effects observed in mice resulted from vaccine administration initiated 7 days after the CEA bearing colon carcinoma cells were transplanted. The antitumor effect should be demonstrated on tumors that have been established for a longer period such as 21 days or more. Another important concern is that the human CEA antigen was used in a murine model. The rationale for the proposed study is to break immune tolerance by immunization with the CEA antigen. The preclinical murine experiments in which immune responses were demonstrated against human CEA. Human CEA is a foreign antigen to mice and may induce an immune response by acting as a hapten, a co-stimulator; therefore, the preclinical experiments are irrelevant.

Dr. Haselkorn asked the investigators to elaborate on an experiment reported by investigators at the Memorial Sloan-Kettering Cancer Institute in which antibody responses to antigens associated with adenocarcinomas were induced by injection of human subjects with chemically treated bovine mucin. Dr. Doi inquired about the level of transgene expression, noting that an expression level of less than 1% is very low.

Dr. Parkman asked the investigators why immunization with plasmid DNA-CEA is expected to be superior to CEA expressed by the vaccinia vector currently under investigation by Dr. Schlom. He noted that Dr. Schlom's vaccinia vector protocol would not have been exempt from RAC review according to the RAC's revised definition of Footnote V-21, exempt vaccines. Dr. Wivel explained that Dr. Schlom's study was initiated before the RAC revised Footnote V-21.

Dr. Post said that the data obtained from the vaccinia-CEA trial should answer the question of whether human CEA can break tolerance and induce an immune response. The investigator has maintained that naked DNA injected into muscle is not integrated within the cell chromosomes, yet CEA has been shown to be persistently expressed for an extended period. Have sensitive experiments been performed to determine the exact percentage of DNA integration? Dr. Parkman asked whether the investigator has access to the data obtained from Dr. Schlom's vaccinia vector trial and whether the use of cytoxan in that trial is important in breaking the immune tolerance by eliminating suppressor cells.

Investigator Responses--Drs. Curiel and Lobuglio

In response to Dr. Haselkorn's concerns, Dr. Curiel stated that the muscle cells are

capable of glycosylating the CEA appropriately and are recognized by a panel of specific antibodies. An immunological response is induced which is the ultimate functional test. Dr. Curiel explained that Dr. Schlom has demonstrated antitumor efficacy of vaccinia-CEA in mice with low tumor burdens.

In response to Dr. Zallen's question, Dr. Curiel said that the RAC has previously approved studies in which injected liposome DNA distributes systemically. Preliminary PCR assays indicate that as few as 5 DNA copies per mg of tissue are detectable one day following injection. DNA was detectable at the injection site, tongue, and gonads of animals. Seven days following injection, DNA was detected only in the tongue. This result suggests that if DNA localizes beyond the injection site, there is no persistent expression. In regard to transduction frequency, Dr. Curiel explained that the 1% frequency described in the protocol is based on published data and not derived from experiments conducted in his laboratory.

In response to Dr. Post's concerns, Dr. Curiel stated that consensus in the literature seems to be that the basis of persistence is not integration. Mixing experiments involving integrated and nonintegrated genes have been conducted by John Wolfe and published data demonstrates that a single integrated copy can be detected in 10^4 to 10^5 cells. Dr. Post stated that although he was unaware of the sensitivity data referred to by Dr. Curiel, a low level of DNA integration should be an acceptable risk.

Dr. Lobuglio of the University of Alabama explained that Dr. Schlom's Phase I vaccinia-CEA trial involves subjects with high tumor burden. There was no evidence of toxicity up to 10^7 virus particles per site. Immunologic response data is not yet available. Dr. Lobuglio stated that he has been contracted by Dr. Schlom's group to conduct a second vaccinia-CEA trial involving subjects with low tumor burden. This second trial will include cytoxan administration and establish evidence of immune response. The basis for the proposed plasmid DNA study is preliminary animal data demonstrating that CEA is immunogenic and can safely produce both immunoprotection and therapy.

Dr. Lobuglio explained that the monoclonal antibodies have been directed to the peptide portion of the molecule. The murine model demonstrated that intramuscular injection of this polynucleotide vaccine resulted in an immune response to the human CEA antigen. The presumption is that the human molecule contains the epitopes that are the target of the immune response.

The issue of breaking tolerance by immunizing an individual with a tolerant antigen is an overriding issue in tumor immunology. Data does not exist relating to the question of CEA immunogenicity in humans. Published data indicate that an immune response to a fetal antigen (not CEA) has been observed in humans.

The term "vaccine" has been changed to "immunization" and "augmentation" throughout the protocol in response to concerns about vaccines inferring disease prevention. There is increasing evidence in the literature that post tumor immunization can produce a detectable immune response.

Dr. Lobuglio explained that Dr. Schlom administered vaccinia-CEA to mice 7 days following tumor cell implantation because tumor growth was rapid enough to become palpable and reach end stage in 28 days. Conversely, 10 to 14 days are required for gene expression by muscle cells *in vivo*. This time-frame between vaccine administration and tumor inoculation was a major constraint to testing this therapy.

In addressing the RAC's concern about compensation for research-related injury, Dr. Lobuglio stated that "all of the normal responsible issues are in place" regarding compensation for negligence. Only compensation for complications arising from the research are excluded. This exclusion will be clearly presented to all subjects.

In response to concerns about possible toxicity of circulating CEA and its immune complexes, Dr. Lobuglio said that no evidence of toxicity has been observed in subjects with circulating CEA receiving up to 1 gram of anti-CEA monoclonal antibody. One of the endpoints of the proposed study is to evaluate tissue injury resulting from an immune response to CEA. No such injuries have been observed in the vaccinia-CEA study. The proposed polynucleotide vaccine strategy is to overcome the diminished immune response to immunogens encoded by vaccinia which can occur in subjects previously exposed to vaccinia. In regard to Dr. Haselkorn's question about bovine mucin, Dr. Lobuglio stated that he was not knowledgeable about such data.

Other Comments

Dr. Parkman commented that the RAC has consistently held investigators to the standard that preclinical evidence must be demonstrated on established tumors. The only data presented by the investigator involved a preimmunization model. Dr. Lobuglio agreed but stated that the proposed strategy was similar to that of the vaccinia trials. Dr. Post commented that a murine model may be irrelevant for the proposed study. Dr. Parkman noted cytokine trials in which animals have received both murine and human cDNA's. The investigator has based his hypothesis that this strategy will provide a better immune response than the vaccinia study without providing the necessary preclinical data. Dr. Lobuglio responded that an alternative, not necessarily better, method is proposed to achieve the desired outcome. The only way to evaluate the relative merits of the proposed study is to perform the human experiment. Dr. Parkman reiterated that preclinical evidence has not been provided demonstrating the efficacy of this treatment against metastases, the RAC's usual standard. If such studies are not technically possible, then the RAC should determine the next level of criteria.

Dr. Straus stated that the proposed direct DNA injection approach is inherently safer than the vaccinia approach; however, there is a lack of preclinical data to support this rationale. He recommended that the murine CEA gene should be used in a murine model. Dr. Miller said that although there is a lack of scientific evidence to support the proposal, the lack of data should be weighed against the potential risk from a recombinant DNA aspect. Since the proposed plasmid does not contain any viral elements, there is no danger of recombination. The RAC could reduce their standards for such innocuous trials. Dr. Chase agreed that the major purpose of this committee

was protecting the public from hazards associated with the recombinant DNA aspects.

Dr. Straus recommended that the RAC should proceed cautiously in their deliberation of this new technology, i.e., direct DNA injection into muscle. Though probably a safe strategy, the proposal would be more acceptable with evidence that an immune response can be developed to murine CEA in a murine model. Dr. Smith noted that safety issues may vary depending on the life expectancy of the subject. Dr. Post commented that the treatments that the subjects have already received is far more toxic than the risks posed by the integration of a few DNA molecules.

Dr. Parkman reminded the committee that whatever action it takes on this protocol will have set a precedent. It is important that the RAC remain consistent so that future investigators have a clear understanding of the requirements and the committee maintains credibility. Dr. Walters asked Dr. Parkman to estimate the time that would be required to perform the relevant preclinical murine experiments. Dr. Parkman responded that 6-9 months would be an approximate speculation.

Dr. Lobuglio stated that he was not aware that the CEA gene had been cloned from a murine library. Each mouse strain has different immune responses; therefore, several different strains would have to be evaluated before drawing any conclusions. He noted that the murine model may not be totally predictive of the human experiment. A substantial amount of information exists on the use of this technology to produce immune responses that are efficacious against infectious diseases. Other investigators have demonstrated the antitumor potential of this model by mixing the splenocytes of immunized animals with tumor cells, and injecting them into naive animals. Total protection against tumor growth was observed *in vitro* at a ratio of 1:1, an amazingly high response.

Dr. Haselkorn said that he favors approval of this protocol. He asked the investigators to submit the immunological data derived from this study to the RAC as soon as possible. He said that it is unacceptable that data is unavailable from the vaccinia-CEA study after 20 patients have been entered onto the study.

Dr. Straus said that in absence of preclinical data involving the murine CEA gene or relevant data from animal tumor model, clinical data from the vaccinia-CEA trial would be an acceptable alternative. Dr. Straus said that he cannot recommend approval of this protocol without reviewing these supporting data.

Dr. Saha asked whether the fact that different mouse strains respond differently to CEA is due to major histocompatibility complex (MHC) restriction. Dr. Lobuglio answered that lack of a consistent immune response is one reason that animal models will not be totally relevant to the human study.

Dr. Parkman agreed with Dr. Straus that the clinical data from the vaccinia-CEA study would be acceptable data to support this protocol. Dr. Haselkorn objected to inclusion of this contingency for approval since these data are from other laboratories and beyond

the control of the present investigators. Dr. Haselkorn stated that even if the vaccinia-CEA study fails, the CEA-DNA injection might yield positive results. Ms. Buc agreed with Drs. Parkman and Straus' assertion that this protocol is not approvable without submission of adequate preclinical data.

Dr. Lobuglio explained that he is involved in the vaccinia-CEA study; however, the data is coded and the results are unknown. Dr. Miller maintained that it is illogical for the RAC to contingently approve a protocol based on data obtained from an experiment that was not reviewed by this committee. Dr. Wivel explained that the vaccinia-CEA experiment was considered exempt from RAC review based on the old definition of Footnote V-21 of the *NIH Guidelines*. The recently amended definition of Footnote V-21 would require RAC review of the vaccinia-CEA trial.

Dr. Curiel said that this trial will provide the data regarding the safety of polynucleotide vaccines which hold great promise for the treatment of many infectious diseases.

Dr. Smith asked whether the same preclinical data will be required if the investigators choose to treat patients with a resectable tumor rather than metastatic cancer. Dr. Lobuglio said that it is logical to begin this Phase I toxicity trial in subjects with advanced cancer.

Dr. Straus asked whether evidence of an immune response has been observed in the vaccinia-CEA study. Dr. Lobuglio responded that all of the subjects in the proposed study have advanced cancer; therefore, any immune response observed in the vaccinia-CEA trial is irrelevant for the proposed study. Dr. Straus said that polynucleotide vaccination is a new technology currently being developed for wide applications. Deliberation of the proposed protocol will set a precedent for the review of future protocols. Dr. Straus expressed concern about establishing a precedent for approval without adequate preclinical data.

Dr. Post asked whether an immune response to CEA in cancer patients is an adequate criterion for this study. Dr. Parkman said that a cellular immune response would be an adequate criterion rather than a humoral response alone. Dr. Lobuglio said that the vaccinia-CEA study involves immunological assays for humoral responses only.

Committee Motion

Dr. Haselkorn made a motion to approve the protocol. Dr. Zallen made a friendly amendment to revise the Informed Consent document incorporating the changes suggested by Drs. Haselkorn, Chase, and Zallen. The amendment was accepted by Dr. Haselkorn.

The RAC approved a motion made by Dr. Haselkorn and seconded by Dr. Miller to accept the protocol submitted by Dr. David Curiel of the University of Alabama, Birmingham, Alabama, by a vote of 10 in favor, 4 opposed, and no abstentions. RAC approval is contingent on the review and approval by the primary RAC reviewers of a

revised Informed Consent document (as approved by the Institutional Review Board) incorporating the changes suggested by Drs. Haselkorn, Chase, and Zallen.

IV-B. ANNOUNCEMENT OF FDA SPONSORED MEETING

Dr. French Anderson of the University of Southern California, Los Angeles, California, announced that the FDA would be sponsoring an open meeting on "Production Issues for Human Gene Therapy" to be held immediately after the RAC meeting on June 9, 1994, in the adjacent conference room. The purpose of this meeting is to informally discuss issues surrounding the production of vectors for use in gene therapy protocols with FDA personnel. He said that this meeting would be the first of ongoing discussions between interested parties. This initial meeting is only to establish an agenda for future meetings. If there is sufficient interest, future meetings will be planned to coincide with RAC meetings.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *CLINICAL TRIAL TO ASSESS THE SAFETY, FEASIBILITY, AND EFFICACY OF TRANSFERRING A POTENTIALLY ANTI-ARTHRITIC CYTOKINE GENE TO HUMAN JOINTS WITH RHEUMATOID ARTHRITIS*/DRS. EVANS AND ROBBINS

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Chris Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Parkman explained that there are two forms of interleukin-1 (IL-1), α, and β. IL-1α causes symptomatology associated with infectious or inflammatory diseases, and attracts granulocytes to the site of inflammation. The body also produces a natural IL-1 receptor antagonist protein (IRAP) which neutralizes the effect of IL-1α on its cellular receptors. So there is considerable research interest in attempts to relieve the IL-1α symptomatology either by systemic administration of IRAP to patients or by administration of gene modified cells producing IRAP. Three areas of clinical or preclinical studies have been performed involving IRAP. The first area is the treatment of sepsis caused by gram negative bacteria or endotoxin shock syndrome. The results of these human trials have not been optimistic. The second area is prevention of graft-versus-host (GVH) disease. Animal studies have yielded optimistic results involving bone marrow transplantation. The third area is the treatment of chronic inflammatory diseases such as rheumatoid arthritis; these studies have been met with varying degrees of success. Most IRAP studies have been conducted by industry, and the data has not been published yet in peer-reviewed journals.

The investigators have constructed an MFG-based retrovirus vector that encodes the genes for human IRAP and herpes simplex virus thymidine kinase (HSV-TK). The investigators propose to generate synovial fibroblasts from the knuckle joints of patients with rheumatoid arthritis who are scheduled to undergo surgery, transduce the fibroblasts with the IRAP vector, inject the transduced cells into the synovial space, and collect

synovial fluid and joint material 1 week later to determine the presence and location of the transduced synovial fibroblasts and the level of IRAP in the joint fluid. This proposal is based upon the demonstrated efficacy of IRAP administration in either human clinical trials or preclinical animal studies.

The investigators indicated in their written response that they are not unswervingly committed to IRAP as the only antiarthritic agent in the human trial. Once the gene transfer techniques have been established, these methods can be employed to transfer genes other than IRAP. The proposed study is similar to a gene marking protocol in that a therapeutic outcome is not expected. He asked the investigators to clarify whether it is intended to be a marking protocol or a study with therapeutic intent since the criteria for approval will be different.

Dr. Parkman said that three outstanding issues remain: (1) Are the high titer vector producer cells described in the protocol currently available for the proposed study? (2) What is the transduction efficiency and the level of IRAP production in the proposed target cells? (3) If the present study is intended as a therapeutic protocol rather than a marking study, histopathologic data should be provided demonstrating the therapeutic effect, i.e., prevention of joint destruction in a rabbit model.

Review--Dr. Motulsky

Dr. Motulsky said that the proposed study is an innovative approach attempting to treat autoimmune disease in the affected joints of chronic arthritis patients. Current treatment is symptomatic and unsatisfactory. Although the IRAP gene is proposed for the current study, the investigators have reserved the option to use other cytokine genes if this procedure is successful. He asked the investigators to respond to the following questions: (1) What is the frequency of severe reactions? (2) What systemic or local reactions to IRAP have been observed that might cause patients to withdraw from the study? (3) What is the status of the experimental animal models described by Dr. Parkman? Preclinical data has been submitted in an antigen-induced arthritis rabbit model. The animals are presensitized to ovalbumin by intradermal injection and then given an intraarticular injection of ovalbumin in the knee joints to induce arthritis. What additional procedures will be required for this study that are not normally performed on patients with advanced knuckle joint arthritis? Dr. Motulsky recommended that a research clinical rheumatologist should be involved in this study to assist in patient evaluation and follow-up. The investigators agreed to include such an expert in this study. Dr. Motulsky recommended approval of this protocol if the requested information is provided.

Review--Dr. Zallen

Dr. Zallen explained that the investigators had already responded to several aspects of the protocol addressed in her written comments. She then raised the following additional questions: (1) Is the viral genetic material spread beyond the joint, specifically to gonadal tissue? This issue is of less concern in the proposed study since it

involves only post-menopausal women; however, such information will have greater significance if this treatment is successful and patients of reproductive age will be involved in future studies. (2) The HSV-TK suicide gene has been incorporated in the vector construct as a safeguard. What are the undesirable side effects that will trigger the use of Ganciclovir (GCV) to eliminate the transduced cells? (3) Have the investigators demonstrated their capability of preparing human synoviocytes in a timely fashion for use in this experiment so that patients will not endure any greater risks including pain and discomfort while waiting for such treatment? What is the maximum time allowed for preparation of the gene altered cells? How would a subject be handled as far as the protocol was concerned if this timetable was not met? (4) The investigators' response indicates that a neutral person will be involved in the informed consent process, but the presence of such a person is not clearly indicated in the Informed Consent document. She stated that the changes suggested for the Informed Consent document have been incorporated, and the investigators have clarified that the costs of the research and any research-related injury will be covered.

Other Comments

Dr. Walters summarized the written comments submitted by Dr. Brinckerhoff. Dr. Brinckerhoff states that the synovial fibroblasts of young rabbit knee joints are easier to grow in tissue culture than the diseased tissues from knuckle joints of older subjects. There is a finite number of fibroblast doubling from cells obtained from human joints. Growing 1×10^7 synovial cells from tissue obtained from a single knuckle joint of an older patient with end-stage joint disease will be technically challenging and difficult to transduce. What is the transduction efficiency in the target cell population? Dr. Brinckerhoff commented that it is unreasonable to evaluate efficacy based on data obtained from a 7-day experiment with fibroblasts obtained from a patient with chronic disease.

Dr. Walters summarized Ms. Meyers' written comments regarding the Informed Consent document. Several specific language changes were suggested to clearly describe the experimental nature of the treatment, recommendations regarding barrier contraception, and request for autopsy.

Dr. Parkman asked whether biochemical data is available regarding the chronic phase of the joint disease in the rabbit model. Dr. Straus was concerned that the IRAP gene introduced into the joints to interrupt the IL-1 mediated inflammatory reaction might diminish the joint's inherent anti-bacterial capacity in the event of a septic joint infection. This risk is more of a concern if the IRAP gene persists in surrounding tissues following removal of the joint. Individuals undergoing this type of surgery with severe rheumatoid arthritis are at particular risk for developing serious infection as a complication.

Dr. Miller raised several questions regarding the vector construct. The vector sequences provided by the investigators are those of the MFG backbone, not the insert. The proposed construct encodes genes for both IRAP and HSV-TK. Only the IRAP construct was used for the rabbit experiments. He suggested that the IRAP construct

should be used for the human experiments in order to avoid possible complications due to immunogenicity of HSV-TK.

Dr. Parkman said that the HSV-TK gene is used for two purposes in this experiment: (1) to eliminate the transduced cells in the event of severe local reaction, and (2) as a surrogate marker to monitor transgene expression. Dr. Miller said that if the HSV-TK gene is to be used in the human study, the same vector construct should be tested in rabbit experiments. Dr. Miller asked the investigators to clarify whether the proposed MFG vector has the frame shift mutation that prevents viral *gag* gene expression or is it the original vector that expresses *gag*.

Dr. Walters remarked that the proposed study does not involve a life threatening disease. Dr. Parkman said that this protocol involves the first autoimmune disease study reviewed by the RAC.

Investigator Responses--Drs. Evans and Robbins

Dr. Evans said that the investigators are well aware that the proposed trial is for a chronic non-fatal disease; therefore, the safety issues are an overriding concern. For this reason, the HSV-TK gene was included as a means to eliminate the transduced cells (particularly for later trials that will last more than 1 week) in the event that the cells escape from the synovial space or cause an unexpected adverse effect. If the RAC considers inclusion of the HSV-TK gene unnecessary, the IRAP alone construct will be used for the human study. He noted that the IRAP construct was used for the preclinical rabbit experiments that demonstrated efficacy.

Dr. Evans presented expression data using the IRAP alone construct versus the IRAP/HSV-TK construct. A preliminary experiment with human synovial fibroblasts (passages 2 and 4) demonstrated between 20 and 50 ng of IRAP/1 x 10^6 cells/24 hours. This level of expression can be further boosted by multiple rounds of transduction. The current expression level exceeds the minimal level of 10 ng/1 x 10^6 cells/24 hours that resulted in a measurable response in rabbits. Dr. Parkman inquired about the amount required for maximum protection and whether the response is measured biochemically, i.e., glycosaminoglycan (GAG) release from the cartilaginous matrix (the presence of IRAP strongly protects the cartilage from breakdown). Dr. Evans responded that maximal protection is observed at 100 ng/1 x 10^6 cells/24 hours, and the response monitored is GAG production.

Dr. Evans presented additional data on GCV sensitivity of HSV-TK transduced cells. Responding to the question of immunogenicity of HSV-TK, he said that HSV-TK is expressed as a intracellular protein and should not be immunogenic. GCV administration is included as a precaution against toxicity from the treatment. GCV administration has been considered for every patient upon completion of the experiment. Dr. Straus was concerned about the rationale regarding the use of GCV, and he said that GCV can cause bone marrow toxicity, particularly in subjects who have bone marrow suppression. Dr. Bill Macaulay of the University of Pittsburgh (Dr. Evans' colleague)

explained that at peak levels, GCV ranges between 4.5 and 10 μg per ml and that 12.5 μg per ml causes bone marrow toxicity. Dr. Evans commented that GCV use is not needed for the present short term experiment of 1 week.

Dr. Evans justified the use of IRAP based on its safety profile in human trials. There has been no toxicity or immunosuppression associated with the use of IRAP. Murine studies were conducted involving MFG-IRAP transduced hematopoietic stem cells that were transferred to syngeneic irradiated host animals. No toxicity was observed in animals that expressed between 2 and 300 ng per ml of human IRAP for life. Upon subsequent challenge with human IL-1, a blocking response was observed. IRAP works well *in vivo* with rabbits and *in vitro* with mouse cells. Dr. Straus asked whether IRAP expression was sustained in the murine experiment. Is there a diminished response to pathogen challenge? Dr. Evans responded that these experiments have not been performed.

Responding to Dr. Miller's question about the MFG vector, Dr. Robbins said the proposed vector includes a frameshift mutation that blocks viral *gag* protein expression. The vector has previously been referred to as MFG-C, but is indicated as MFG in the present protocol.

Responding to Dr. Parkman's question regarding protection of the joint from tissue destruction, Dr. Evans explained that GAG release into the synovial fluid has been used to measure joint cartilage destruction. In the rabbit experiments, IRAP gene transduction blocked GAG release. Dr. Parkman agreed that these biochemical data reflect acute phase joint destruction, but a more relevant model is the effect of IRAP on the chronic phase of the disease. Dr. Evans said that the proposed 7-day study is not for the purpose of achieving clinical improvement, but to answer the question of whether the transduced IRAP gene is biologically active in an arthritic joint. Therefore, a more sensitive biochemical response has been chosen for this study. Dr. Evans said that most clinical studies on IRAP have been conducted by Synergen (Boulder, Colorado), and most of these data has not been published. The present study is independent of Synergen. Dr. Evans emphasized that the primary purpose of the study is to use the IRAP gene to develop a gene delivery system for treating joint diseases. If positive results are obtained, the same system can be adopted for future efficacy trials.

Committee Motion

Dr. Parkman made a motion to approve the protocol using the vector that does not contain the HSV-TK gene and submission of additional experiments on transduction efficiency. Dr. Evans concurred. Dr. Miller made a friendly amendment that safety data should be provided demonstrating no adverse effects of transduced cells outside the joint tissues. Dr. Parkman accepted the amendment.

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Zallen to accept the protocol submitted by Drs. C. H. Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, by a vote of 13 in favor, 0 opposed, and 1

abstention. RAC approval is contingent on the following: (1) the vector construct encoding the HSV-TK gene will not be used, (2) submission of data (a minimum of 4 reproducible experiments) demonstrating efficient transduction of human synovial cells with the vector that will be used in the human protocol, and (3) submission of safety data demonstrating that transduced synovial cells expressing IRAP do not traffic to other sites following intra-joint injection.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *USE OF A RETROVIRAL VECTOR TO STUDY THE TRAFFICKING PATTERNS OF PURIFIED OVARIAN TIL POPULATIONS USED IN INTRAPERITONEAL ADOPTIVE IMMUNOTHERAPY OF OVARIAN CANCER PATIENTS: A PILOT STUDY*/DR. FREEDMAN

Review--Dr. Dronamraju

Dr. Walters called on Dr. Dronamraju to present his primary review of the protocol submitted by Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas. This protocol is a resubmission of the proposal that was deferred by the RAC at its June 7-8, 1993, and March 3-4, 1994, meetings. The RAC deferred the protocol at its March 1994 meeting until the investigator returned to the full RAC with the following: (1) a modified protocol that includes a revised treatment schema, and (2) a revised Informed Consent document that describes the clinical procedures to be performed in language that is understandable to laypersons.

Dr. Dronamraju said that most of the previous questions raised by the reviewers have been answered by the investigator. The investigator has agreed to choose 1 month after infusion of the marked tumor infiltrating lymphocytes (TIL) as the time point for marker detection rather than 2 to 3 months as previously proposed. In response to Dr. Brinckerhoff's previous concerns, the investigator has clearly defined a successful outcome of gene marking. The specific uptake of marked TIL at the tumor sites will be supported when the mean numbers of marked cells in tumor tissue is significantly larger than the mean number of marked cells in the normal tissues. Dr. Dronamraju asked the investigator to clarify the meaning of their response to Dr. Brinckerhoff's review that "5% of the infected cells being marked, 9 evaluable patients will provide meaningful answer." In response to Dr. Brinckerhoff's written comments, the investigator has submitted polymerase chain reaction (PCR) assay data demonstrating his ability to detect 1 neomycin resistance (*neo*^{R}) marked cell in 1 x 10^5 cells.

Dr. Dronamraju noted Ms. Meyers' written suggestion that the Informed Consent document should be revised such that patients will not be expected to pay for any research-related costs. The investigator indicated in his written response that the difficulties encountered previously in transducing CD8(+) cells have been overcome. Overall, Dr. Dronamraju said that all reviewers are in agreement that most questions have been answered satisfactorily.

Review--Dr. Brinckerhoff (presented by Dr. Dronamraju)

Dr. Brinckerhoff's written comments were presented by Dr. Dronamraju. Dr. Brinckerhoff states that the investigator has adequately responded to the issues previously raised by the RAC; therefore, approval of the protocol is recommended. Dr. Brinckerhoff recommended that for future protocols, investigators should present their protocol in a logical and thoughtful manner with supporting data and sufficient detail.

Review--Dr. Secundy (presented by Dr. Dronamraju)

Dr. Dronamraju summarized Dr. Secundy's written comments regarding the Informed Consent document. Dr. Secundy recommended that language should include the provision of medical care for any adverse effect that may possibly result from the treatment.

Other Comments

Dr. Zallen commented on the inconsistency of statements regarding medical costs in the Informed Consent document. Item 11, which appears to be a standard statement required by the Institutional Review Board (IRB) of MD Anderson Cancer Center, stated that if the investigational agents become commercially available during the course of this study, the patients may be required to cover the cost of subsequent doses. The document states that costs related to medical care, including expensive drugs, tests, or procedures, shall be the patient's responsibility unless other funding sources contribute toward the costs. This statement is inconsistent with that in item 3, i.e., that there is no cost to the patients for the preparations of cells, viruses, or laboratory tests. Dr. Zallen asked the investigator to clarify this inconsistency. Dr. Walters added that this same concern was raised in Ms. Meyers' written comments.

Dr. Parkman asked whether the ongoing TIL trials would be discontinued if this marking study demonstrates no selectivity or specificity of the TIL cells in tumor versus normal tissue.

Investigator Response--Dr. Freedman

Responding to Dr. Dronamraju's question regarding 5% marking of infected cells, Dr. Freedman said 5% refers to the dilution effect. The TIL cultures are expanded in 2 bioreactors at a transduction rate of 10%; therefore, each bioreactor will have 5% of the transduced cells. Regarding the cost of medical care, subjects will not be required to pay for such costs; however, the MD Anderson IRB insists that the statement in item 11 be included. Dr. Zallen said that inclusion of two inconsistent statements in the Informed Consent document is unacceptable. The costs and risks that are unique should be stated explicitly for each protocol rather than inclusion of a general statement in all Informed Consent documents. Dr. Freedman responded that he will request that the IRB address this issue. Dr. Parkman commented that there are no unique risks associated with this marking protocol that do not apply to the ongoing TIL trial except for the *neo*^{R} marker gene. Dr. Chase agreed with Dr. Zallen's concern about the contradictory statements in the Informed Consent document. Dr. Walters suggested inclusion of the following

statement in item 11: "Please see item 3 for costs that will not be charged to you in this study." Ms. Buc said that the statement as written in item 11 is uninformative as to covered costs and is inconsistent with the statement in item 3.

Responding to Dr. Parkman's question regarding the possibility of a negative outcome of this study, i.e., non-specific trafficking of TIL to tumor, Dr. Freedman stated that the TIL trial will continue regardless of the outcome of the marking study. Dr. Parkman said that conducting a study that will not impact the future course of investigation is inconsistent with the strategy of conducting clinical research. Dr. Chris Platsoucas of Temple University (a co-investigator) explained that mechanisms other than TIL cell trafficking, e.g., enhanced cytokine production by TIL may contribute to the antitumor effect; therefore, the TIL trial should continue regardless of the outcome of the marking study.

Dr. Post inquired whether this protocol would have been considered exempt under the *Accelerated Review* process. Dr. Wivel responded that this protocol is a resubmission of a protocol that was deferred by the RAC; therefore, the *Accelerated Review* guidelines are not applicable.

Committee Motion

Dr. Dronamraju made a motion to approve the protocol with a request to resolve the discrepancy regarding items 3 and 11 of the Informed Consent document as previously suggested by Dr. Zallen.

The RAC approved a motion made by Dr. Dronamraju and seconded by Dr. Post to accept the protocol submitted by Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas, by a vote of 13 in favor, 1 opposed, and no abstentions.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *USE OF DOUBLE MARKING WITH RETROVIRAL VECTORS TO DETERMINE THE RATE OF RECONSTITUTION OF UNTREATED AND CYTOKINE EXPANDED CD34(+) SELECTED MARROW CELLS IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION*/DRS. HESLOP, BRENNER AND KRANCE

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Childrens Research Hospital, Memphis, Tennessee. Dr. Miller explained that autologous bone marrow transplantation (ABMT) is often used in pediatric patients to allow the administration of high dose chemotherapy. Bleeding and infection are risks often associated with ABMT. To improve ABMT methods, the marrow is often pre-incubated with growth factors that increase the rate of engraftment. If engraftment can be significantly enhanced, smaller amounts of marrow may be harvested in the future. The

investigators propose to determine whether the addition of growth factors enhances engraftment and persistence of the engrafted cells. Two portions of marrow will be marked with *neo*^{R} using two distinguishable retrovirus vectors, LNL6 and G1Na. One portion of the cells will be treated with growth factors and the other will remain untreated. Both portions of cells will be reinfused at the time of transplant. The time and persistence of engraftment will be determined.

Dr. Miller said that the proposed protocol is similar to other marking protocols previously approved for the same laboratory. The investigators are competent and experienced. He recommended approval of this protocol. He noted that the Institutional Biosafety Committee (IBC) recommended Biosafety Level (BL) 2 physical containment for the retroviral vectors, but BL1 is sufficient according to the *NIH Guidelines*.

Review--Dr. Chase

Dr. Chase recommended that a statistician should be consulted for data analysis and accurate determination of the growth pattern differences between treated and untreated cells across a heterogeneous pool of subjects.

Review--Ms. Buc

Ms. Buc commented on minor inconsistencies in the Informed Consent document as follows: (1) All items in the "statement of understanding" are not included in the Informed Consent document, and (2) the statement "I know my or my child's records will not be given to anyone outside the hospital unless I agree" is not necessarily true as these records could be subpoenaed. She commended the investigators for writing such an understandable Informed Consent document.

Dr. Walters commented that this Informed Consent document could serve as a model for other investigators. He noted Ms. Meyers' written comments suggesting that recommendations for long-term follow-up and a request for autopsy should be included in the Informed Consent document.

Ms. Buc said that the proposed study promises to definitively answer important questions regarding bone marrow engraftment and holds the promise for improving current therapy methods.

Investigator Response--Dr. Heslop

Dr. Heslop agreed to incorporate minor changes in the Informed Consent document suggested by Ms. Buc.

Dr. Miller noted the IRB's provisional approval of the protocol pending RAC review. Are there other issues raised by the IRB that have not been addressed? Dr. Heslop answered that there are no outstanding issues; provisional approval refers to informing

the IRB of any changes in the protocol required by the RAC. Dr. Post commented that this protocol would have qualified for the *Accelerated Review* process.

Committee Motion

The RAC approved a motion made by Dr. Miller and seconded by Ms. Buc to accept the protocol submitted by Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Childrens Research Hospital, Memphis, Tennessee, by a vote of 14 in favor, 0 opposed, and no abstentions.

VIII. WORKING GROUP ON DATA MANAGEMENT--SEMI-ANNUAL DATA MANAGEMENT REPORT/DR. SMITH

Dr. Smith summarized the semi-annual data reports submitted by the PIs of NIH-approved human gene transfer protocols. A total of 219 patients have been entered on NIH-approved human gene transfer studies (68 subjects since the last reporting period). Of the 72 protocols recommended for approval by the RAC, 63 have been approved by the NIH Director, 39 are considered active, 10 are considered closed, 21 are pending FDA approval, and 9 are pending NIH Director approval (due to failure of the investigators to meet the RAC's stipulation requirements for approval).

A new column has been added to the Semi-Annual Data Management Report entitled, "Biological Efficacy." This column is applicable only to gene therapy protocols. The term "published" is entered in this column for protocols in which demonstration of biological efficacy has been published in a peer-reviewed journal.

The "*In Vivo* Evidence of Gene Transfer" column has been modified to include additional information regarding gene expression. For the gene marking studies, information will be given to indicate detection of marked cells in the recipients. For gene therapy protocols, information will be collected regarding detection of the transgene in recipients' cells and expression of the gene product. Such information is irrelevant for vaccine protocols.

Dr. Smith made the following recommendations regarding future semi-annual data reports: (1) demonstration of the virus or gene product in a site other than the target should be reported as an "adverse effect", (2) investigators should indicate the number of subjects who have undergone autopsy and provide relevant data, and (3) a letter should be sent to investigators who have not enrolled any subject onto the protocol for several years inquiring whether the study should be considered "closed."

Dr. Chase asked about the compliance rate for data reporting. Ms. Debra Wilson of the NIH Office of Recombinant DNA Activities (ORDA) said that the response rate is over 90%. Dr. Ross asked if information regarding the number of eligible patients identified versus the number of subjects entered. Dr. Smith said that with one exception (Dr. Deisseroth's study) such information is not requested or available.

Dr. Smith pointed out one discrepancy regarding patient enrollment in the protocol by Dr. Galpin, et. al. (Protocol #9306-048). This protocol was submitted for RAC review on a voluntary basis since NIH funding was not involved. The protocol has accrued 16 patients while the RAC approved a maximum of 15 subjects. No explanation has been provided for over-accrual. Drs. Parkman and Straus said that Viagene, Inc., should be notified since it is the sponsoring company and bears partial responsibility for data reporting. Ms. Buc said that if the protocol is not obligated to have RAC review under the *NIH Guidelines*; therefore, data reporting should be collected on a voluntary basis. Dr. Post concurred with Ms. Buc's statement. Dr. Zallen said that the investigators have benefitted from the RAC review and approval process; therefore, data reporting should be required. Dr. Chase said that future studies submitted on a voluntary submission should be approved with the stipulation that data reporting is mandatory. Ms. Wilson stated that the results obtained from this trial, i.e., immunological assays, may have bearing on future protocols submitted by the same sponsoring company. Dr. Noguchi agreed with the statement made by Ms. Wilson. Dr. Noguchi stated that the FDA encourages companies to voluntarily submit their protocols for RAC review, otherwise, public review by FDA committees would be required.

Ms. Sheryl Osborne of Viagene, Inc., responded that the immunological data from Dr. Galpin's trial is contained in a coded format; therefore, the results are not yet available. Ms. Osborne stated that some of the subjects enrolled in this trial are on the placebo arm; perhaps, accounting for the discrepancy in patient number.

Dr. Smith pointed out an adverse event report dated March 31, 1994, submitted by Dr. Oldfield for a subject entered on the HSV-TK/GCV glioblastoma study (Protocol #9206-019). A hemorrhagic complication occurred as a result of multiple intratumoral injections (44). The adverse event was not directly related to gene transfer. Dr. Walters said the RAC will follow up on this event. The other significant adverse effect was observed in Dr. Crystal's cystic fibrosis (CF) study (Protocol #9212-034). Dr. Wivel said that one subject developed pneumonitis related to the high dose of adenovirus vector. The investigators have reduced the vector dose.

Summarized below are the categories of human gene transfer protocols that have been approved by the RAC to date:

	T	M	Total (T + M)
RAC Approved	50	22	72
NIH Director Approved	41	22	63
Categories of RAC-Approved Protocols			
Cancer	32	4	36
Cystic Fibrosis	7	0	7
SCID/ADA	1	0	1

Acute Hepatic Failure	0	1	1
Familial Hypercholesterolemia	1	0	1
Gaucher Disease	3	0	3
Bone Marrow Marking/Cancer	0	15	15
HIV(+)	5	2	7
Alpha-1-Antitrypsin Deficiency/Acute Lung Injury	1	0	1

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *USE OF SAFETY-MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF BREAST CANCER: A PILOT TRIAL*/DRS. DEISSEROTH, HORTOBAGYI, CHAMPLIN, AND HOLMES

Review--Dr. Brinckerhoff (presented by Dr. Dronamraju)

Dr. Walters called on Dr. Dronamraju to present the primary written review of Dr. Brinckerhoff for the protocol submitted by Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas. Dr. Brinckerhoff stated that this study is based on Protocol #9306-044, previously approved by the RAC for the treatment of ovarian cancer. This proposal differs in three aspects: (1) patient eligibility is limited to breast cancer not ovarian cancer; (2) peripheral blood mononuclear cells will be transduced with the gene for multidrug resistance type-1 (MDR-1), not autologous bone marrow cells; and (3) the peripheral blood cells will undergo immunohistochemical analysis to rule out the presence of contaminating breast cancer cells.

A retrovirus vector encoding the MDR-1 gene will be used to transduce autologous peripheral blood hematopoietic stem cells. Prior to transduction, these stem cells will be cryopreserved while the patient undergoes chemotherapy. Following chemotherapy, 50% of the CD34(+) cells will be thawed, transduced with the MDR-1 vector, and infused into the patient. The MDR-1 marked cells will be monitored by means of a methylcellulose late progenitor colony culture system and a PCR assay for the MDR-1 gene. The acquisition of chemotherapy resistance by stem cells will be monitored using culture assays by drug selection. These studies will evaluate whether the introduction of the MDR-1 gene into peripheral blood CD34(+) cells confers chemotherapy resistance; therefore, allowing for a more intensive Taxol regimen. Ultimately, increased doses of chemotherapeutic agents may lead to improved prognosis for breast cancer patients.

Dr. Brinckerhoff stated that this proposal is a logical extension of Dr. Deisseroth's ongoing ovarian cancer protocol (Protocol #9306-044). Although this protocol may have qualified for the *Minor Modification* process, a change in the tumor target raises a

number of important questions that should be addressed; therefore, approval as a minor modification is not appropriate. The investigators proposed transduction of peripheral blood mononuclear cells rather than bone marrow cells in order to reduce the probability of transducing contaminating cancer cells in the marrow. What is the probability of tumor cell contamination? In their written response, the investigators stated that the immunohistochemical assay is capable of detecting 1 contaminating cancer cell in 100,000 normal cells. The investigators provided published data on assay sensitivity. Dr. Deisseroth was asked to expand on the ability of his laboratory to perform these assays at the same level of sensitivity. Dr. Brinckerhoff asked the investigators to respond to the following questions during his oral presentation: (1) Is the transduction rate of peripheral blood mononuclear cells as efficient as that of bone marrow cells? (2) Will increased doses of Taxol exceed the capacity of the MDR-1 transduced cells to confer resistance? Dr. Brinckerhoff stated that if satisfactory responses are provided to all of these concerns, approval of the protocol is recommended.

Review--Dr. Dronamraju

Dr. Dronamraju expressed concern about the Informed Consent document statement, "costs related to my medical care including expensive drugs, tests or procedures... required by this clinical research study shall be my responsibility." This statement poses ethical and moral dilemmas and will preclude participation if the patient's financial status changes during the course of their participation in the study. Dr. Dronamraju inquired about the transduction efficiency. In the investigator's written response, they stated that the transduction rate for peripheral blood cells is as efficient as bone marrow cells. The investigators anticipate that approximately 50% of the subjects entered on the study will complete the protocol. Patients will be followed systematically for 5 years.

Review--Ms. Meyers (Presented by Dr. Dronamraju)

Dr. Dronamraju summarized the written comments submitted by Ms. Meyers on the Informed Consent document. Ms. Meyers suggested the following: (1) patient follow-up should be for life rather than for 5 years, (2) the vector manufacturer should be added as a party who may access patients' medical records, and (3) patients who are poor or uninsured should not be excluded from participation in the protocol. In the investigators' written response, they stated that MD Anderson Cancer Center will provide free care to uninsured residents of Texas.

Other Comments

Dr. Parkman asked about patient accrual in the ovarian cancer protocol (Protocol #9306-044). Dr. Smith asked if there is an exclusion criterion based on a defined number of contaminating breast cancer cells in the marrow.

Investigator Response--Dr. Deisseroth

Dr. Deisseroth explained that patients entered on this study will have Stage III/IV breast cancer and failed initial therapy but have not received Taxol. Subjects will have negative bone scans and negative marrow involvement as demonstrated by microscopic and immunohistochemical methods. The immunohistochemical assays are sufficiently sensitive to detect one contaminating cancer cell in 1 x 10^6 normal cells. The combination of patient eligibility criteria and assay sensitivity will ensure that the MDR-1 chemoprotection gene will not inadvertently transduce tumor cells. Prior to the collection of peripheral blood cells, subjects will receive a dose of cyclophosphamide to reduce the number of circulating cancer cells. CD34(+) selection of peripheral blood cells will further preclude the possibility of contaminating cancer cells since the latter are CD34(-). The proposed strategy of this study is that introduction of the MDR-1 chemoprotection gene into CD34(+) peripheral blood cells will allow delivery of high dose Taxol post-ABMT to eradicate breast cancer cells.

Dr. Deisseroth stated that the 10% transduction efficiency observed in CD34(+) peripheral blood cells is equivalent to the transduction efficiency of CD34(+) bone marrow cells. Responding to the question of possible cancer cell contamination, he responded that the immunohistochemical assay used to screen patients is sensitive enough to easily identify patients who have cancer cells circulating in their peripheral blood. The bone marrow will be assayed for contaminating cancer cells. Since bone marrow has a contamination rate 1,000 times higher than that of peripheral blood mononuclear cells, a negative assay demonstrated on bone marrow cells reduces the probability of peripheral blood contamination to less that 1 in 1 x 10^8 cells. CD34 selection should further reduce this possibility to less than 1 in 1 x 10^9 to 1 x 10^{10} cells. Since a maximum of 2 x 10^7 cells will be transduced, the chance of transducing the MDR-1 gene into a single cancer cell is extremely small. Any possible risk to patients is insignificant compared to the potential for benefit. He expects that 20 patients entered on the study should yield 10 evaluable patients, i.e., participation through completion of the study.

Dr. Deisseroth responded to questions regarding the Informed Consent document. Patients will be monitored for life. MD Anderson Cancer Center will provide free medical care to uninsured patients who are residents of Texas. For those patients who have insurance, financial counselling will be provided to ensure that there is sufficient insurance coverage before admission to the protocol. Research-related costs will not be charged to patients; these costs are provided by research grants. Dr. Chase expressed concern about contradictory statements in the Informed Consent document regarding disclosure of financial obligations. Dr. Deisseroth responded that the Informed Consent document will be revised to clarify this issue and submitted to the IRB.

Dr. Holmes commented that the IRB has formed a subcommittee specifically to address the issue on providing mechanisms for payment of medical costs that are not considered as routine patient care. Dr. Chase applauded this effort by the MD Anderson IRB and said that letters should be sent to other IRBs to urge similar action. Dr. Wivel noted that such an action is beyond the purview of the RAC.

Dr. Deisseroth said that no patients have been entered on the ovarian cancer protocol because FDA approval is pending.

Dr. Richard Cote of the University of Southern California (co-investigator) explained the immunohistochemical assay to detect contaminating cancer cells in peripheral blood and bone marrow samples. The test employs a mixture of monoclonal antibodies directed to cell surface glycoproteins and cytoskeletal elements of cells of epithelial origin including breast and ovarian carcinoma cells. He described spiking experiments involving a mixture of breast cancer and normal bone marrow or peripheral blood CD34(+) cells. At varying concentrations, between 2 and 5 tumor cells were detectable among 1×10^6 normal cells. The presence of breast cancer cells in bone marrow is biologically relevant since patients are more likely to have recurrence at this site following chemotherapy. Dr. Deisseroth reiterated that screening patients for bone marrow metastases is a major safety feature.

Dr. Deisseroth described murine experiments in which CD34(+) cells were transduced with MDR-1. These mice were resistant to Taxol at a maximum dose of 30 mg/kg body weight. This dose is equivalent to the highest dose proposed for the human study; therefore, the same degree of safety is expected for the human protocol. In response to Ms. Meyers' written comments, Dr. Deisseroth said that patients will undergo life-long follow-up. There is no manufacturer involved in the vector production; the vectors will be produced in the investigators' laboratory.

Dr. Anderson commented on his personal experience regarding negotiation of medical coverage with the local IRB. IRB negotiation involves the IRB as well as the hospital administrators, board of trustees, and legal counsel. Ms. Buc responded that extensive negotiation is required only for deciding who is responsible for such costs. The issue of Informed Consent document clarification merely involves an amendment. Such amendments are easily dealt with at the IRB level. Dr. Deisseroth noted that most patients entered on this study have failed numerous other treatments. The "spirit" of the Informed Consent document is disclosure, i.e., possible unforeseen complications not directly related to gene therapy. Medical care will be provided to subjects independent of their economic status.

Committee Motion

The RAC approved a motion made by Dr. Dronamraju and seconded by Dr. Parkman to accept the protocol submitted by Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas, by a vote of 13 in favor, 0 opposed, and no abstentions.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *RETROVIRAL MEDIATED GENE TRANSFER OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C GENE TO HEMATOPOIETIC PROGENITORS OF GROUP C PATIENTS*/DRS. LIU AND YOUNG

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Johnson M. Liu and Neal S. Young of NIH, Bethesda, Maryland. This protocol is a resubmission of the proposal that was deferred by the RAC at its December 2-3, 1993, meeting. The RAC deferred the protocol until the investigators returned to the full RAC with the following: (1) murine data demonstrating *in vivo* expression of the Fanconi anemia complementation group C (FACC) gene and safety data accumulated over a period of ≥ 4 months demonstrating that the FACC-transduced cells do not result in any untoward effects; (2) data as cited in Dr. Cynthia Dunbar's semi-annual data report (Protocol #9206-025) regarding the possibility that "stem cell factor could favor the growth of leukemic versus normal progenitors during *ex vivo* culture periods"; and (3) revision to both the protocol and Informed Consent document to modify the eligibility criteria regarding the necessity for bone marrow examination following each reinfusion.

Dr. Parkman said that this protocol is a meritorious study. Preclinical studies demonstrate that *in vitro* transduction of the FACC gene into lymphoblast cells of Fanconi anemia patients results in the normalization of sensitivity to mitomycin C treatment and a reduction in the number of induced chromosomal breaks. Two outstanding questions remained following the previous RAC review: (1) What are the long-term effects (≥ 6 months) of the FACC transduced gene in murine bone marrow cells? and (2) Does the presence of granulocyte colony stimulating factor (G-CSF) during the transduction procedure promote the outgrowth of preleukemic cells? Dr. Cynthia Dunbar (Protocol #9206-025) previously reported that G-CSF provided a growth advantage to chronic myelogenous leukemia (CML) cells. In response to these questions, the investigators concluded: (1) published literature demonstrates that the administration of G-CSF to Fanconi anemia patients does not increase the probability of developing myeloid malignancy. (2) Data published by Dr. Cynthia Dunbar indicates that although the presence of the transmembrane form of the stem cell growth factor promotes a selective growth advantage to CML cells, the soluble form of this factor has no deleterious effect. The proposed transduction procedure includes the soluble form of the stem cell growth factor rather than the transmembrane form; therefore, selective growth advantage is not a safety concern. The investigators have provided long-term toxicology data derived from murine experiments demonstrating that no lymphoproliferative abnormality has been observed for a period of 6 months. Dr. Parkman stated that the RAC's previous concerns have been satisfactorily answered by the investigators; therefore, approval of the protocol is recommended.

Review--Dr. Smith

Dr. Smith agreed that most of the RAC's concerns have been addressed by the investigators. The question of stem cell factor in the transduction procedure has been addressed. The data demonstrated that this factor's effect is specific to CML cells whereas normal progenitors are not as sensitive. Although one may disagree as to the relative merits of various components of the growth factor cocktail to be used in the transduction procedure, the investigators have now provided sufficient reassurance to

allow the proposed transduction procedure. The investigators have provided adequate data in response to toxicology questions relating to the long-term effect in an animal model. He recommended approval of the protocol.

Other Comments

Dr. Post asked the following questions: (1) Was the transduced FACC gene continuously expressed during the course of 6 month murine experiments? (2) Was the FACC gene expressed in cell colonies growing out of the transduced human CD34(+) peripheral blood cells?

Investigator Response--Dr. Liu

Dr. Liu responded to several issues regarding quantitation of FACC RNA. FACC RNA was detected by the reverse transcriptase polymerase chain reaction (RT-PCR) assay of murine peripheral blood cells. Northern Blot analysis demonstrated low level FACC expression that was detectable at the end of the 6 month murine experiment. DNA PCR was used to demonstrate the presence of the transgene in human bone marrow cells. A functional assay was employed to determine improved colony growth. Data was presented on 3 Fanconi anemia cell lines representing 3 different mutations of the FACC gene. Normalization of mitomycin sensitivity was demonstrated by transduction of FACC gene into the cell line containing a stop codon mutation of the FACC gene. The corrected Fanconi CD34(+) cells had a growth advantage over the defective cells, a fact that would favor engraftment of the transduced cells. Data was presented demonstrating transgene expression of transduced lymphoblast cell lines as well as cells of Fanconi anemia patients. The investigators looked at this sensitivity to mitomycin C in terms of chromosomal aberration and found that after gene correction, creation of cytogenetic abnormalities was reduced to normal. Data indicates that FACC is not an oncogene but plays a role in DNA repair. Fanconi anemia cells have an unusually prolonged G2 cell cycle phase. Introduction of the FACC gene into these cells diminishes the chance that cells with unrepaired DNA will enter into mitosis.

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Smith to accept the protocol submitted by Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, by a vote of 13 in favor, 0 opposed, and no abstentions.

XI. AMENDMENT TO PART I-D OF THE *POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA INTO THE GENOME OF ONE OR MORE HUMAN SUBJECTS (POINTS TO CONSIDER)* OF THE *NIH GUIDELINES* REGARDING INFORMED CONSENT/DR. ZALLEN

Dr. Zallen provided background information about the efforts of the Working Group on

Informed Consent regarding revision of Part I-D, Informed Consent, of the *Points to Consider*. At the December 2-3, 1993, RAC meeting, Dr. Gary Ellis, Director of the NIH Office for Protection from Research Risks (OPRR), suggested that many of the RAC's concerns regarding informed consent could be addressed by amending the *Points to Consider*. At the March 3-4, 1994, RAC meeting, the working group presented two draft versions of revised Part I-D of the *Points to Consider* for RAC consideration. Following a lengthy discussion, the RAC recommended that elements of the two draft documents should be incorporated into a single document for review and approval by the RAC. Following a working group telephone conference, a revised document was developed.

Part I-D-1 of the revised document requests information regarding the informed consent process. Part I-D-2 describes issues that should be addressed in the Informed Consent document. Part I-D-2-a describes general requirements for research involving human subjects. Part I-D-2-b describes special requirements specific to gene transfer research, i.e., use of barrier contraception, long-term follow-up, request for autopsy, and protection from the media.

Dr. Motulsky commented on Part I-D-2-a-(5) which includes verbal descriptions of quantitative information relating to risk. The proposed language is inconsistent with the common usage of the terms *rare*, *uncommon*, *common*, and *frequent*. The proposed document defines *rare* as < 0.1%; *uncommon* as < 1%; *common* as 1 to 10%; and *frequent* as > 10%. Dr. Motulsky explained that the more common usage of these terms is as follows: *rare* as < 0.5%; *uncommon* as 0.5 to 2%; *occasional* as 2 to 15%; *common* as 15 to 35%, and *frequent* as > 35%. Dr. Parkman expressed concern about assigning numerical values to verbal descriptors since most risks, i.e., adverse events, cannot be precisely quantitated due to the extremely small number of patients who have been entered onto gene transfer trials to date. Ms. Buc said that the use of verbal descriptors is acceptable if used to disclose the investigators' quantitative estimate of risks. Dr. Smith agreed with Dr. Motulsky's quantitative definition. Dr. Chase said that quantitative information can be determined from an appropriate probability model; therefore, the verbal descriptors are acceptable as defined. Dr. Walters asked the RAC whether the proposed terms should be associated with a numerical value. Drs. Smith and Miller emphasized that quantitative definition are preferable. Ms. Buc said that investigators retain the option not to include these terms in their Informed Consent documents.

Dr. Zallen said that Ms. Lori Andrews (a RAC member who was not present) suggested revision of the autopsy section to indicate that permission for autopsy will be requested but it is not a requirement.

Regarding Part I-D-2-b-(1) on barrier contraception, Ms. Buc said that it is inappropriate to exclude pregnant women from participation in gene transfer studies since it assumes that a woman's right is subservient to that of the fetus. Dr. Zallen said that this exclusion is to protect the gene therapy research from being associated with any untoward effect such as a malformed newborn even if it is not directly due to gene

transfer. Dr. Walters commented that this exclusion will prevent inadvertent gene transfer to the fetus. Dr. Parkman added that the recommended duration of barrier contraception should be specified. Dr. Chase said that this exclusion is a balance between the females right to treatment versus the desire to protect the nascent field of gene transfer research. Dr. Motulsky concurred with Dr. Chase's comments.

Regarding the statement, "In the consent form, subjects should be informed about...any costs for medical treatment...as a *direct* result of research-related injury", Dr. Parkman said that the word *direct* is problematic based on variable interpretation and recommended that the term *direct* be deleted. Ms. Buc suggested that the phrase, "In the consent form" is redundant and should be deleted.

Dr. Walters suggested that the RAC empower Dr. Zallen and the ORDA staff to incorporate the suggested editorial changes.

Ms. Buc reiterated her concern about excluding women from participation in Phase I/II gene therapy trials since such studies are often the last hope of benefit for terminally ill patients. Dr. Chase said that the issue is the right of pregnant women to a treatment versus a collective right to safeguard a new form of medical experimentation. Both men and women are urged to practice barrier contraception with no reference to gender. Dr. Parkman suggested that the statement, "female subjects should be informed..." should be eliminated to avoid gender discrimination. Dr. Miller noted that most investigators would be reluctant to perform such a novel treatment on pregnant women. Ms. Buc stated that due to the controversial nature of this issue, the RAC should not take a position on the exclusion of pregnant women. Drs. Ross and Smith said that participants should be informed of the possible risk rather than outright exclusion. Dr. Parkman asked whether the FDA has a policy on this issue. Dr. Noguchi said that the FDA would not dismiss such a protocol outright unless there was clear scientific reason as to why women of child bearing age or pregnant women should not be included in the protocol. Dr. Parkman concluded that the investigators should: (1) describe whether pregnant women will be included in the study, and if not, provide an explanation for their exclusion, (2) describe possible risks to the fetus, and (3) include specific recommendations regarding contraception. Dr. Parkman suggested a new title for Part I-D-2-b-(1) entitled: "Reproductive Considerations." Dr. Noguchi said that Dr. Parkman's recommendations are consistent with FDA policy.

Committee Motion 1

The RAC approved a motion made by Dr. Chase and seconded by Dr. Haselkorn to accept the proposed amendments to Part I-D, Informed Consent, of the *Points to Consider* by a vote of 13 in favor, 0 opposed, and no abstentions. These amendments were approved with the exception of Parts I-D-2-b-(1) (Barrier Contraception) and I-D-2-b-(3) (Request for Autopsy), deletion of the words "in the Informed Consent document" where appropriate, and the inclusion of minor editorial changes.

As to the use of verbal descriptors for possible risks, Dr. Chase suggested that if these

descriptors are used to convey quantitative information, such terms should be thoroughly explained to the patients.

Committee Motion 2

The RAC approved a motion made by Dr. Chase and seconded by Dr. Motulsky to amend Part I-D-2-a-(5) (Possible Risks, Discomforts and Side Effects) by a vote of 13 in favor, 0 opposed, and no abstentions. The following sentence will be added to the current language:

> "If verbal descriptors (e.g., *rare*, *uncommon*, or *frequent*) are used to express quantitative information regarding risk, these terms should be explained."

Further discussion ensued regarding proposed language for Part I-D-2-b-(1) regarding contraception. Dr. Parkman summarized the conclusions as follows: (1) the subject will be informed concerning the possible risks of the experiment to the fetus, (2) the subject will be informed concerning the necessity for barrier contraception during the active phase of the experiment, (3) the investigator will define the duration of the active phase of the experiment, and (4) the inclusion/exclusion of pregnant women in the experiment should be addressed. The working group will make minor editorial changes as necessary.

Committee Motion 3

The RAC approved a motion made by Dr. Parkman and seconded by Ms. Buc to amend the title of Part I-D-2-b-(1) by a vote of 13 in favor, 0 opposed, and no abstentions. Part I-D-2-b-(1) will be entitled *Reproductive Considerations*.

XII. DELETION OF APPENDIX L, *RELEASE INTO THE ENVIRONMENT OF CERTAIN PLANTS*, OF THE *NIH GUIDELINES*/DR. WIVEL

In a letter dated April 29, 1994, Dr. Nelson A. Wivel, Executive Secretary, RAC, requested that Appendix L, *Release into the Environment of Certain Plants*, be deleted from the *NIH Guidelines*. Dr. Wivel stated that Appendix L should be deleted based on the following: (1) Section I of the *NIH Guidelines* allows experiments to proceed that are reviewed and approved by another Federal agency that has jurisdiction for review and approval without the necessity for NIH review or approval (except for experiments involving human subjects) (52 FR 31849); (2) the RAC has not reviewed any deliberate release experiments involving recombinant DNA since 1984; (3) at its May 30-31, 1991, meeting, the RAC recommended deletion of Section III-A-2 of the *NIH Guidelines* regarding environmental release; and (4) experiments involving deliberate release into the environment are currently reviewed within the framework of existing Federal regulations, i.e., the Environmental Protection Agency and the United States Department of Agriculture.

The RAC approved a motion made by Ms. Buc and seconded by Dr. Chase to delete Appendix L from the *NIH Guidelines* by a vote of 13 in favor, 0 opposed, and no

abstentions.

Appendix L will be deleted as follows:

"APPENDIX L. RELEASE INTO THE ENVIRONMENT OF CERTAIN PLANTS

"Appendix L-I. General Information

"Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Working Group, and specific approval by the NIH Director. Such experiments also require the approval of the IBC before initiation. Information on specific experiments which have been approved will be available in ORDA and will be listed in Appendix L-III when the Guidelines are republished.

"Experiments which do not meet the specifications of Appendix L-II fall under Section III-A and require RAC review and NIH and IBC approval before initiation.

"Appendix L-II. Criteria Allowing Review by the RAC Plant Working Group Without the Requirement for Full RAC Review.

"Approval may be granted by ORDA in consultation with the Plant Working Group without the requirement for full RAC review (IBC review is also necessary) for growing plants containing recombinant DNA in the field under the following conditions:

"Appendix L-II-A. The plant species is a cultivated crop of a genus that has no species known to be a noxious weed.

"Appendix L-II-B. The introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants.

"Appendix L-II-C. The vector consists of DNA: (i) From exempt host-vector systems (see Appendix C); (ii) from plants of the same or closely related species; (iii) from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants; (iv) from plant pathogens only if sequences resulting in production of disease symptoms have been deleted; or (v) chimeric vectors constructed from sequences defined in (i) to (iv) above. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests.

"Appendix L-II-D. Plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance

with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review by the IBC. Copies must also be submitted to the Plant Working Group of the RAC."

XIII-A. AMENDMENT TO PART VI OF THE *POINTS TO CONSIDER* OF THE *NIH GUIDELINES* REGARDING EXPEDITED REVIEW OF SINGLE PATIENT HUMAN GENE TRANSFER PROTOCOLS/DR. WIVEL

In a letter dated April 29, 1994, Dr. Nelson A. Wivel, Executive Secretary, RAC, requested that Item #4 of Part VI, *Expedited Review of Single Human Gene Transfer Patients*, of the *Points to Consider*, should be amended to clarify submission requirements for *Expedited Review*.

The *Procedures to be Followed for Expedited Review* currently reads:

> "4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols.

The proposed amendment reads:

> "4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols; therefore, submission of the *Points to Consider* is required."

The RAC approved a motion made by Ms. Buc and seconded by Dr. Chase to amend Part VI of the *Points to Consider* regarding expedited review of single patient human gene transfer protocols by a vote of 13 in favor, 0 opposed, and no abstentions.

XIII-B. CHAIR REMARKS

Dr. Walters noted that there is an expanding volume of information that is currently published regarding human gene therapy. He noted two new journals in this field, i.e., *Gene Therapy* and *Cancer Gene Therapy*, in addition to *Human Gene Therapy* which is now published monthly. Dr. Parkman remarked that NIH has expanded its funding of research programs involving human gene therapy. One such example comes from the National Heart, Lung, and Blood Institute which is requesting gene therapy proposals for the treatment of heart and cardiovascular diseases. The National Institute of Child Health and Human Development has recently requested applications for prenatal gene therapy research proposals for the treatment of degenerative central nervous system diseases.

XIV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN

GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I TESTING OF GENETICALLY ENGINEERED INTERLEUKIN-7 MELANOMA VACCINES*/DRS. ECONOMOU, GLASPY, AND McBRIDE

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California. This proposal is an open label, Phase I clinical trial to evaluate the safety and immunological effects of administering IL-7 producing allogeneic and autologous melanoma cells to patients with metastatic melanoma. Three groups of 5 patients will receive 1 x 10^7 irradiated, unmodified autologous tumor cells mixed with increasing numbers of IL-7 transduced allogeneic melanoma cells (M24 cell line). The number of transduced cells will be adjusted to produce increasing doses of IL-7 between 10 and 1,000 ng/24 hours. The vaccine will be administered as 3 biweekly subcutaneous inoculations. The study design will allow a dose-escalation evaluation of local and systemic toxicity. A final group of 10 patients will receive 3 biweekly inoculations of their autologous melanoma cells transduced with an IL-7 retrovirus vector. Antitumor immunity will be measured by tumor cell skin tests, tumor biopsies, generation of specific antibodies for autologous tumor and M24 cells, and generations of cytotoxic T lymphocyte (CTL) precursors.

Dr. Motulsky noted that this is the first proposal involving the IL-7 transgene in human subjects. The investigators have submitted both *in vivo* and *in vitro* experiments demonstrating an antitumor effect against melanoma cells. Data indicates stable cytokine expression and induction of CTL in mixed lymphocyte tumor cell cultures. The investigators are currently conducting similar studies using the IL-2 transgene; therefore, they possess sufficient expertise to conduct the study. The retrovirus vector will be produced and tested by Targeted Genetics, Inc., Seattle, Washington.

Review--Dr. Doi

Dr. Doi asked the following questions: (1) Has the IL-7 transduced M24 cell bank been certified by the FDA? (2) Have rodent and monkey experiments been conducted to determine IL-7 toxicity? (3) The doses of IL-7 to be administered to patients are 10, 100, and 1,000 ng/24 hr. Since the maximum obtainable level of IL-7 production is 10 ng IL-7/1 x 10^6 cells/24 hr, 1 x 10^8 transduced M24 cells would have to be administered to achieve the highest dose in addition to the autologous cells. Is this large number of cells feasible? (4) Have high dose IL-7 producer cells been successfully cloned? (5) Should the HSV-TK gene be deleted from the vector construct to prevent possible complications? (6) Is quantitative data available demonstrating the superiority of IL-7 over IL-2, IL-3, IL-6, and tumor necrosis factor?

Review--Ms. Meyers (presented by Dr. Walters)

Ms. Meyers raised three issues of concern in her written comments: (1) the use of the

term "vaccine" throughout the protocol and the Informed Consent document, (2) recommendations for barrier contraception only for females and not for males, and (3) the legitimacy of approving simultaneous studies involving multiple cytokines.

Other Comments

Dr. Smith asked if the proposed dose of irradiation sufficiently prohibits proliferation yet maintains adequate IL-7 production.

Dr. Parkman asked the following questions: (1) What are the results of the previously RAC-approved melanoma protocol involving IL-2? (2) Is there any data derived from preclinical animal experiments demonstrating an antitumor effect against preexisting tumor? Such a model would be more clinically relevant than this 10 day preimmunization model.

Dr. Zallen asked how the investigators would avoid conflict of interest in eliciting informed consent from subjects for whom they provide care. Is there a match between the human leukocyte antigen (HLA) locus of the subject and the proposed cell line?

Drs. Dronamraju and Chase asked how the investigators arrived at the proposed number of 5 patients per dose escalation group. Dr. Parkman asked the investigators to justify their choice of IL-7 over IL-3 since the data indicates that IL-3 is more effective in the preimmunization model. Dr. Smith inquired whether the same subjects will be targeted as the ongoing IL-2 trial (Protocol #9309-058). If the same individuals are targeted, how will decisions be made regarding assignment of subjects to individual protocols?

Investigator Responses--Drs. Economou and Overell

Dr. Economou stated that Dr. Robert Overell of Targeted Genetics Corporation, Seattle, Washington, would respond to questions regarding cell bank certification, toxicology studies, and inclusion of the HSV-TK gene. In response to Dr. Doi's question regarding the autologous arm of the study, Dr. Economou said that a success rate of 90% has been achieved in establishing primary cultures from melanoma patients. Transduction of these cells results in a level of IL-7 production between 10 and 50 ng IL-7/1 x 10^6 cells/24 hours. There will be no attempt to clone high producing autologous cells. 1,000 ng IL-7/1 x 10^7 cells/24 hours will be the upper limit for IL-7 production. Bulk transduced cells will be selected for hygromycin resistance and are expected to produce IL-7 within the range of between 10 and 50 ng/24 hours. In the allogeneic arm of the protocol, doses of 10, 100, and 1,000 ng IL-7/24 hours will be achieved. The immunological response will be determined and compared to the autologous tumor cell data in which IL-7 production is variable.

Regarding Ms. Meyers' concerns, Dr. Economou stated that both male and female patients would be counselled with regard to contraception. Premenopausal women must have had a negative pregnancy test prior to entering the study.

Responding to Dr. Smith's question, Dr. Economou said that data on the effects of irradiation on cytokine production has been published in the journal *Blood* (reprint was appended in the submission). The M24 cell line transduced with the IL-7/HyTK retroviral vector has stable IL-7 production over 7 days after 10,000 cGy irradiation. Dr. McBride stated that 10,000 cGy is more than adequate to inhibit growth of these tumor cells. Dr. Smith asked if similar data is available for the primary autologous tumor cells and if these cells are clonogenic following irradiation? Dr. Economou responded that stable IL-7 production was maintained for greater than 7 days but clonogenic assays were not performed. M24 was selected because this cell line has been administered to hundreds of patients in polyvalent allogeneic vaccine trials at the University of California, Los Angeles (UCLA). For these previous vaccine studies, 5,000 cGy irradiation was used and no evidence of tumor growth was observed at this dose.

In regard to the results of the IL-2 melanoma study, Dr. Economou explained that the IL-2 trials were delayed due to a change in the vector supplier. Therefore, both the IL-2 and IL-7 protocols will be conducted in parallel which would allow for comparisons between the two studies. Both studies are similar in design.

Dr. Economou explained that the animal studies are difficult to perform using a preexisting tumor model because these tumors are anaplastic and grow so rapidly that the animals die within 3 weeks. There is insufficient time to generate systemic tumor specific immunity to control the tumor growth. Perhaps the model system could be modified to allow a longer preexisting period and reducing the inoculating tumor dose; however, such a modified system would not reflect the clinical situation. He stressed that the human study is the only real test of this treatment in which safety and immunity will be determined.

Dr. Parkman stated that the lack of adequate preclinical data is a major weakness in this proposal. The previously approved IL-2 study was based on substantial preclinical data in an animal model that demonstrated an effect on metastatic tumors. IL-7 is a new cytokine and the scientific basis for this study is clearly inadequate. IL-3 is superior to IL-7 in preimmunization model experiments. What is the rationale for choosing IL-7? Dr. Economou answered that both IL-3 and IL-7 demonstrate the most significant cytokine effect in animal models. IL-3 was not chosen for this study because its biology is not well understood. IL-7 is more of a T cell specific cytokine. CD4 and CD8 cells are largely the cells that are attracted to tumor sites. Dr. Economou explained that his laboratory has been studying the effect of IL-7 transduced cells on melanoma for several years. Tumor infiltrating lymphocytes isolated from IL-7 transduced tumors demonstrate tumor specific cytotoxicity. Dr. Parkman suggested the possibility of using the B16 melanoma model since B16 was the preexisting tumor model used for the IL-2 preclinical studies.

Dr. Overell questioned whether the RAC has maintained consistency in its expectations regarding preclinical data. Dr. Parkman said that the RAC has consistently required demonstration of an antitumor effect in a preexisting tumor model. Clear rationale for therapeutic benefit should be demonstrated in preclinical studies even for a Phase I

toxicity study. Dr. Post stated that it may be preferable to examine the data as a whole and determine if there is clear rationale for the proposal rather than defining specific experiments. Dr. Parkman expressed concern that IL-7 has never been administered to humans; therefore, toxicity is unknown. Dr. Chase stated that there is no statistical rationale for the proposed study. What conclusions can be drawn from a study involving 5 patients in each experimental group?

Dr. Walters asked Dr. Noguchi to comment on this issue. Dr. Noguchi responded that the 219 patients entered in RAC approved trials to date do not provide a sufficient base by which to address common toxicity, i.e., 1 in 100 subjects. The primary goal of a Phase I study is to estimate toxicity. Toxicity in drug trials is usually not observed until the Phase II study. He said that 5 subjects per group is preferable to 3, particularly for rare genetic diseases.

Dr. Economou explained that he has consulted with Dr. William Cumberland, Professor of Biostatistics at UCLA School of Public Health, regarding statistical analysis and the size of the treatment groups. Because of the number of variables in the proposed study, the number of subjects to be entered in each group is statistically difficult to determine. It is the opinion of the investigators that 5 subjects would yield useful data regarding toxicity and immunological responses.

Responding to Dr. Smith's question on patient selection, Dr. Economou explained that metastatic melanoma patients who have failed conventional treatment are referred to UCLA and are eligible for several protocols that are being conducted simultaneously. Usually subjects are triaged with at least 2 cycles of chemotherapy. Subjects with subcutaneous or regional disease are then offered the option of entering one of the adoptive immunotherapy trials. Subjects that are not entered on an adoptive immunotherapy study are offered other systemic trials. The vaccine trials are offered as a possible third level of treatment. Entry on one research protocol does not preclude entry into another study. Some subjects can be entered onto more than one study in a sequential fashion. Dr. Haselkorn recommended that a patient advocate should be assigned to assist subjects in understanding the studies and making informed decisions.

Dr. Chase stated that the choice of the number of patients based on budget and duration of a protocol is a reasonable one if there is no other statistical criteria. Entering subjects sequentially on studies could lead to unrecognized consequences that could cloud results. Dr. Economou responded that melanoma patients usually demonstrate very clear responses to beneficial treatment. Given sufficient time between entry on individual protocols it is possible to determine the effectiveness of each treatment. The major objectives of the present study are toxicity and immunological endpoints which are easily discernible.

Dr. Parkman asked about the specific criteria for entering patients on either the IL-7 or the IL-2 protocol. Dr. Economou responded that subjects will be assigned in a random fashion. Dr. Chase commented that randomization of patient entry is an acceptable procedure.

In response to Dr. Haselkorn's suggestion regarding assigning a patient advocate, Dr. Economou stated that neither he nor Dr. Glaspy are of the opinion that a neutral person is necessary to discuss informed consent with patients. The research is secondary to patient care and all subjects receive comprehensive information about all research protocols for which they are eligible. Subjects are frequently referred to studies other than the adoptive immunotherapy trials.

In response to Dr. Zallen's concerns, Dr. Economou explained that although patients are HLA typed, an HLA match is unnecessary because the M24 cell line is used for cytokine production and not antigen presentation. Dr. Overell explained that M24 has been used in a number of clinical trials at UCLA. This cell line is part of a biologics master file that Targeted Genetics, Inc., will be filing with the FDA. Validation tests are outlined in the submission materials. With regard to toxicology studies, experiments are currently being conducted in monkeys and mice. In the monkey model, animals receive recombinant IL-7. In the murine experiment, animals receive multiple administration of irradiated transduced tumor cells. Both of the ongoing animal models will be evaluated for evidence of toxicity. Dr. Parkman asked whether there is data documenting IL-7 induced abnormalities in transgenic mice, e.g., vitiligo (development of white patches in skin), or other autoimmune phenomena as a result of responses to the melanin within these cells. If such an observation would occur, toxicities should be disclosed in the Informed Consent document.

Dr. Overell responded to Dr. Parkman's question on the rationale for the starting dose of 10 ng IL-7/24 hours. This dose is based on data derived from murine experiments that included doses between 10 and 100 ng/24 hours. Dr. Parkman expressed concern about whether the murine data is an adequate basis for the rationale. The norm for other cytokine studies is to determine doses by extrapolation of data from human systemic toxicity studies. Dr. Post inquired if the monkey and murine toxicity studies have been completed. Dr. Overell said that these studies have recently been initiated. The monkey study involves doses 100 times over the dose proposed for the human trial. The murine studies will be used to test for autoimmune disease. Dr. Parkman commented that these toxicity data are needed for writing a scientifically based Informed Consent document. Dr. Smith added that it will be difficult to approve the protocol without these toxicity data. Dr. Parkman stressed that this protocol is the first application of IL-7 to human subjects, and the present proposal is not supported by adequate preclinical data. Dr. Noguchi commented that he would agree with Dr. Parkman that FDA would not approve a trial without extensive negotiation regarding the study design and data from toxicology experiments. It is entirely appropriate for the RAC to raise these concerns.

Dr. Motulsky said that considering the lack of toxicology studies, he would recommend deferral of the protocol until the investigators return to the RAC with these data. Dr. Miller asked for clarification regarding the toxicology data that would be required. Dr. Parkman inquired whether IL-4 was given to humans systemically before approval of the IL-4 gene therapy protocol. Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, stated that IL-4 was administered systemically in combination

with IL-2 to 100 patients before the IL-4 gene therapy trial was proposed, but extensive toxicology data in animal models was not required by the RAC. Dr. Parkman commented that this clinical scenario is different in which no human IL-7 data is available. Dr. Overell said that negotiations have already occurred with the FDA regarding the kind of toxicology data that will be required. He asked whether the data required by the FDA would be satisfactory for the RAC. Dr. Wivel commented that the FDA required data will serve the purpose. Dr. Parkman noted that a justification for the starting dose is necessary and the result related to scleroderma. Dr. Walters concluded that the toxicology study design negotiated with FDA will be acceptable to the RAC. Dr. Parkman said that due to the serious deficiency of preclinical data, it would require a new submission for the future RAC review, not just provisions of toxicology data.

Committee Motion

Dr. Motulsky made a motion to defer the protocol. Dr. Parkman made a friendly amendment that efficacy data must be provided from preexisting tumor models when the protocol is resubmitted. Dr. Motulsky did not accept the friendly amendment.

The RAC approved a motion was made by Dr. Motulsky and seconded by Dr. Doi to defer the protocol submitted by Drs. Economou, Glaspy, and McBride of the University of California, Los Angeles, California, by a vote of 12 in favor, 0 opposed, and 2 abstentions. The RAC deferred the protocol based on insufficient toxicology studies and failure to demonstrate biological efficacy. Dr. Parkman recommended that data demonstrating biological efficacy in a preexisting murine tumor model be provided when the protocol is resubmitted. Dr. Motulsky said that he would not require such data. Dr. Walters noted that Dr. Miller abstained from voting due to his collaboration with Targeted Genetics, Inc..

Chair Note

Dr. Walters welcomed Dr. David Ginsberg of the University of Michigan as a new member of the RAC and Dr. Harold Ginsberg as an *ad hoc* consultant for the adenovirus protocol submitted by Dr. Jack Roth of MD Anderson Cancer Center, Houston, Texas.

XV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *CLINICAL PROTOCOL FOR MODIFICATION OF TUMOR SUPPRESSOR GENE EXPRESSION AND INDUCTION OF APOPTOSIS IN NON-SMALL CELL LUNG CANCER (NSCLC) WITH AN ADENOVIRUS VECTOR EXPRESSING WILDTYPE p53 AND CISPLATIN*/DR. ROTH

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. Jack A. Roth of M.D. Anderson Cancer Center, Houston, Texas. Dr. Haselkorn explained that this protocol involves the *in vivo* transduction of wildtype p53,

a tumor suppressor gene, into lung cancer cells to inhibit cell division, i.e., prevent tumor growth. The p53 protein forms complexes with normal transcriptional factors that control the expression of other genes that promote cell division through the activation of DNA synthesis. Cells do not divide in the presence of functional wildtype p53. In the absence of wildtype p53, cells divide in an uncontrolled manner. A substantial number of NSCLC have been shown to have the defective p53 gene. The investigator proposes to replace the defective gene with the corrected p53 gene to inhibit tumor cell division. Murine and human lung cells have been transplanted into nude mice and this strategy has been demonstrated to be functionally effective.

This human protocol involves the delivery of the wildtype human p53 gene to lung tumors. The delivery process involves modifying an adenovirus such that early genes required for early transcription and DNA replication are replaced with a human wild type p53 gene. The adenovirus vector, Ad5CMV-p53, will be injected into NSCLC patients.

The investigators have demonstrated that the addition of cisplatin at the time of transduction by the adenovirus-p53 construct induces apoptosis (programmed cell death). Cisplatin alone has been shown to reduce growth of tumor cells in culture. The p53 construct by itself effects a 28% reduction in the cell growth. However, the combination of cisplatin and the p53 adenovirus construct have a synergistic effect. This phenomenon forms the scientific basis of the human protocol.

Dr. Haselkorn raised the following questions: (1) What is the scientific explanation for the synergistic effect of cisplatin and p53? (2) Since this protocol requires injection into a defined tumor, why is the tumor not surgically removed? He said that most of the previous concerns raised in the primary written review have been adequately addressed by the investigators.

Review--Dr. Straus (presented by Dr. Haselkorn)

Dr. Haselkorn summarized Dr. Straus' written comments. Dr. Straus stated that the combined treatment of the p53 gene and cisplatin was a potentially exciting Phase I study. The investigators propose 2 study groups with up to 21 patients in each group. One group will receive direct injection of the adenovirus construct directly into the tumor mass. The second group will have the adenovirus construct injected into the pleural space (for patients with a pleural disease). Dr. Straus suggested that subjects should receive either the adenovirus vector or cisplatin alone as a preliminary toxicity study prior to administering the combination. The current 2 week window between administration of the adenovirus alone and its use in combination with cisplatin may not be adequate to assess toxicity and the potential for adenovirus replication and shedding. Immunosuppression caused by cisplatin may augment virus shedding. He suggested that a background rate for virus shedding should be determined, and the treatment groups and total number of subjects should be reconsidered with this aspect in mind.

Dr. Straus suggested that the Informed Consent document should be revised with the

following modifications: (1) the statement that the adenovirus vector "cannot cause disease" should be deleted, (2) the incomplete sentences should be corrected, and (3) the ambiguous statements regarding medical costs should be revised such that these issues are clearly disclosed. Dr. Straus concluded that approval is recommended contingent on incorporation of the suggested revisions.

Review--Mr. Capron (presented by Dr. Haselkorn)

Dr. Haselkorn stated that Mr. Capron provided a very detailed written analysis of the protocol. Mr. Capron states that the preclinical studies sufficiently justify the human trial. The injection of the recombinant adenovirus does not pose an unacceptable risk to other individuals who may come in contact with these subjects. Mr. Capron raised several questions about the number of patients entered on the study and the statistical design. He recommended several changes in the Informed Consent document for clarification.

Review--Ms. Meyers (presented by Dr. Haselkorn)

Dr. Haselkorn presented Ms. Meyers' written concern about the Informed Consent documents regarding medical costs and life-long follow-up. Ms. Meyers questioned whether 21 patients are justified for this Phase I trial.

Review--Dr. H. Ginsberg (Ad hoc)

Dr. H. Ginsberg commented on the biology of the adenovirus construct. One advantage of the proposed construct is that the inserted p53 gene replaces the E1b viral gene, thus preventing the complex formation between p53 and the 55 kilodalton E1b protein. He noted; however, that the cell transduction at a high multiplicity of infection may augment the expression of early genes. This vector is not a totally defective virus. He expressed concern that the pathogenicity of the virus has not been considered. Although viral DNA expression is required for the pathological response, the vector can produce severe pathogenic effects in cotton rats, mice, and non-human primates. Pathogenicity must be assessed prior to administering this construct to patients with pulmonary disease. One advantage of this vector over previous adenovirus vectors used in CF protocols is that the early region 3 (E3) has been retained that helps protect the cells from cytopathic effects.

In regard to the issue of potential pathogenicity, Dr. H. Ginsberg explained that some animal models do not demonstrate clinical signs of adenovirus infection similar to humans; therefore, the choice of an animal model is important. The cotton rat is the model of choice because clear pathogenic effects of adenovirus infection are evident. Adenovirus pathogenic effects do not require viral replication. If large doses of adenovirus vector are administered, expression of early genes can produce such effects in the absence of viral replication. Pathogenicity is first observed in the alveoli followed by peribronchial and perivascular infiltration. Epithelial cells are not affected; therefore, epithelial cell brushings are inadequate to test for pathogenicity. There should be histologic examination of the tissue. Because the vector does not replicate, large doses

of the vector, i.e., between 1 x 10^9 and 1 x 10^{10} plaque forming units (PFU), would be required to determine pathology in the human lung.

Other Comments

Dr. Doi noted the adverse event reported by Dr. Ronald Crystal (Protocol #9212-034) with regard to adenovirus vector administration. Dr. H. Ginsberg said that the major pathological response is the result of early cellular immunity, predominantly CTL responses. Deletion of E1a does not prevent early gene expression, a fact that will limit multiple inoculations of adenovirus vectors. Dr. Haselkorn commented that the adverse event reported for Dr. Crystal's CF study was related to target cell destruction resulting from an antibody response to late viral proteins. Since this proposal involves the destruction of tumor cells, such an effect could be beneficial. Dr. Haselkorn asked the investigators to elaborate on this concern. Drs. Miller, Parkman, and Haselkorn asked Dr. Roth to address the phenomenon of the "bystander effect."

Dr. Miller asked about the dose of virus proposed for the human study and whether assays have been performed to determine the presence of helper virus. Is sequence data available for the junction points of p53 and the vector backbone? How will the virus be injected into the tumor mass?

Dr. Smith asked how this protocol relates to Dr. Roth's previous NSCLC/retrovirus vector protocol in terms of patient entry. He asked Dr. H. Ginsberg how far the adenovirus vector might be expected to traffic outside the immediate injection area. Dr. H. Ginsberg responded that escape of the vector outside the injection area is unlikely because the transgene will only react within the cell.

Dr. Parkman asked about the percentage of transduced cells necessary to observe the "bystander effect." Has the "bystander effect" been observed with the adenovirus vector as with the retrovirus vector? What percentage of cells is expected to be transduced using this vector *in vivo* or *in situ*?

Dr. Post noted that the protocol calls for the administration of the adenoviral vector alone followed by a combination of the vector and cisplatin. Will the preliminary administration of the vector alone result in an immune response against the second injection? In regard to intrapleural administration, where is the virus expected to target?

Dr. Saha raised a concern that p53 gene mutations could possibly convert the tumor suppressor gene into an oncogene. What is the likelihood of such an occurrence, i.e., p53 undergoes somatic mutation?

Dr. Haselkorn explained the bystander effect is a phenomenon where more cells are killed than are actually infected or transduced by the vectors. One model for this postulates that a diffusible substance is released by dying cells that causes apoptosis of surrounding cells. Dr. Parkman explained that apoptosis is a programmed cell death triggered by either a natural or pharmacological substance.

Dr. H. Ginsberg cautioned against assaying for adenovirus in the supernatant of the transduced cells since infected cells will not be lysed immediately and released into the supernatant. More than 90% of adenovirus is associated with the cell pellets.

Investigator Response--Dr. Roth

Dr. Roth hypothesized about the mechanism for the combined effect of p53 and cisplatin. The function of wildtype p53 is to cause a delay in cell cycle progression that allows for the repair of damaged DNA. If p53 is nonfunctional, the cell will continue to divide and DNA damage will accumulate. Introduction of wildtype p53 into cells damaged by irradiation induces apoptosis (programmed cell death). A similar mechanism might be operative as a result of excessive cisplatin DNA damage. Cisplatin is a DNA crosslinking agent.

In response to the reviewers comments regarding the Informed Consent document, Dr. Roth explained that suggestions have been incorporated to the extent allowed by the IRB. Paragraphs 10 and 11 regarding the compensation for research-related injuries and medical costs including expensive tests or procedures cannot be changed or removed at this time. He stated that he will work with the other MD Anderson investigators and the IRB on this issue.

Dr. Roth explained that resectable tumors will be removed surgically. However, due to invasion of vascular and bronchial structures, not all tumors confined to a local region are surgically resectable. These non-resectable areas, as well as recurrences and isolated metastasis, can be injected by a computed tomography (CT) guided needle. Tumors are usually soft and easily injected. Dr. Roth presented a slide demonstrating the spread of the injected material throughout the tumor mass as indicated by the *lacZ* marker. Distribution of the vector to the peripheral blood circulation is limited.

Dr. Roth addressed the safety features of the proposed vector construct. The cytomegalovirus (CMV) promoter, which expresses p53, is susceptible to inactivation. Therefore, p53 expression is transient, and the p53 protein is no longer detectable 15 days following transduction. If there is any mutation in the p53 gene, the mutant protein would be expressed only for a short time.

Dr. Saha asked if the investigator is capable of detecting a virus with the mutant p53 gene. Dr. Roth presented data from a spiking experiment in which a transforming Abelson leukemia virus was mixed with 1×10^{10} adeno-p53 virus particles. Foci formation was not observed upon infection of NIH3T3 cells. Dr. Miller commented that Abelson virus is a retrovirus, not an adenovirus.

Dr. Roth presented diffusion experiments using the *lacZ* marker gene. Following a single injection, the vector was distributed to over 60% of the tumor cells as estimated by a planimetric measurement. With the bystander effect of the tumor killing activity of the transduced cells, most cells in the tumor mass will be treated. He presented data from the orthotopic lung cancer model to verify that this is a highly reproducible system.

By flushing the tumor sites with the adeno-p53 vector, no evidence of tumor at the p53 treated site was observed after 30 days. Sequential injections were used in the murine model. The first injection of the adeno-p53 construct reduced the growth of the 30-day established tumor. The second injection with the combination of p53 and cisplatin completely inhibited tumor growth and induced apoptosis. These *in vitro* and *in vivo* experiments have been accepted for publication in the *Journal of the National Cancer Institute*.

Patient eligibility will be limited to subjects with local or regionally advanced NSCLC. The intrapleural administration arm of the study involves subjects with malignant pleural effusion who are not eligible for surgery, radiation, or other local treatment. For intrapleural administration, the adenovirus vector will be delivered through a chest tube. Dr. Post asked if adenovirus vectors have been previously delivered to the pleural cavity in any human trial. Dr. Roth answered that human subjects have not received adenovirus vectors intrapleurally to date. Dr. Post explained that unlike intratumoral injection, normal cells in the pleural cavity will be transduced by the adenovirus construct. Dr. Post asked if biopsies of eligible subjects will be screened by PCR for the p53 gene mutation. Dr. Roth responded that demonstration of this mutation is included in the eligibility criteria.

Dr. Roth explained that the rationale for injecting larger tumors and including pleural disease is that a more concentrated adenovirus vector preparation can be obtained than with retrovirus vectors.

Dr. Parkman expressed concern that the transduction of normal cells within the pleural cavity might result in pleuritis or pleurisy. Dr. Post asked if the wildtype p53 gene might render normal cells in the pleural cavity more susceptible to cisplatin. Dr. Parkman asked if mouse or monkey experiments have been performed to determine toxicity of the adeno-p53 construct when injected into the pleural cavity following cisplatin treatment. Dr. Roth responded that these experiments have not been performed. Dr. Parkman said that although the majority of the cells lining the pleural cavity are nondividing, the lining is continuously renewed by the dividing stem cells underneath. Destruction of these stem cells may result in serious pleural disease.

Dr. Roth presented PCR data in response to concerns about wildtype replication-competent adenovirus contamination of the vector preparations. One wildtype virus particle is detectable among 1×10^9 vector particles. The patient dose will range between 1×10^{10} and 1×10^{11} particles; therefore, a maximum of 100 wildtype virus might be expected. One order of magnitude higher (1,000 wildtype virus particles) is required to cause human disease. Dr. Miller commented that a plaque assay is a more biologically relevant assay than PCR.

Dr. Wei-Wei Zhang of MD Anderson Cancer Center (co-investigator) explained that the wildtype helper virus has been assayed by two methods: (1) PCR of the E1a gene, and (2) DNA labeling of HeLa cells infected with the virus. Helper virus was not detected in a background of 1×10^9 vector particles.

Dr. Roth presented slides demonstrating p53 expression with no effect on bronchial epithelial cell proliferation at 7 days. Dr. Haselkorn asked if the experiment has been performed in combination with cisplatin. Dr. Roth responded that the cisplatin experiment has not been performed. Dr. Parkman suggested that the best model for the effect of the combination therapy would be to administer these agents to the pleural cavity of cotton rats.

Dr. Roth presented histological data from Balb/c mice experiments involving intrabronchial administration of adenovirus constructs. At the highest virus dose (1 x 10^{10} PFU), slight evidence of perivascular infiltration was observed with no evidence of pneumonia. Dr. Roth quoted a letter from Dr. James Wilson of University of Pennsylvania stating that no pathogenicity was observed in 8 subjects receiving 5 x 10^{7} PFU of their adenovirus constructs which have both E1 and E3 deletions and are more pathogenic than the proposed construct in which the E3 gene has been retained. Dr. Roth stated that all research-related costs of the trial are underwritten by a sponsored research agreement.

Dr. Roth addressed the possible mechanism of the "bystander effect." Upon transduction with the adeno-p53 vector, molecules are secreted from the transduced cells that affect killing of the nontranduced cells. These protein molecules have a molecular weight in the range between 30 and 100 kilodaltons. These molecules may be released from cells undergoing apoptosis. Dr. H. Ginsberg commented that the E3 gene has been reported to inhibit apoptosis.

Dr. Roth noted that there is no shortage of eligible patients for this study. Eligible patients will be screened through a conference mechanism. Based on these discussions, subjects can make informed decisions regarding protocol participation.

Responding to concerns about sequential administration of the vector, Dr. Roth said that cisplatin could be administered with the first dose of the adeno-p53 vector.

Dr. Parkman asked whether the clinical trial could be separated for the two groups of patients, those with local lesions and those with intrapleural lesions. Additional toxicology experiments should be conducted prior to approval of the intrapleural arm of the study. Dr. Roth agreed that the two arms of the study could be separated. Dr. Parkman favored approval only for the local disease arm of the study.

Dr. Zallen confirmed that as with other MD Anderson protocols, problems remained with Section 11 of the Informed Consent document that requires patients to pay for costs related to medical care. Dr. Roth reiterated his willingness to work on this issue with the IRB. Dr. Parkman suggested that this issue could be clarified by inclusion of a statement that patients will not be responsible for any research-related costs; however, they may be required to cover some of the costs for treatment.

Drs. Parkman and H. Ginsberg suggested cotton rats should be used for the toxicology studies since they are the most sensitive to adenovirus. However, if the investigators still

insist on using mice, the C57/Black strain is preferable.

Dr. Miller raised a concern about spreading of the adenovirus vector carrying a mutated p53 gene with oncogenic potential to cells outside the injected areas or to other individuals. The safety data presented did not rule out the generation of mutant virus in the vector preparations. Dr. Roth disagreed with Dr. Miller's interpretation stating that cells transduced for 2 weeks should not have any oncogenic potential. Dr. Smith suggested that subjects should be monitored for virus shedding. Dr. Miller suggested and Dr. Roth concurred that patient's sputum will be collected and assayed for the presence of adenovirus on 293 cells expressing the viral E1a and E1b genes.

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Haselkorn to accept the protocol submitted by Dr. Jack A. Roth of M.D. Anderson Cancer Center, Houston, Texas, by a vote of 11 in favor, 1 opposed, and 1 abstention. Approval of the protocol is contingent on the review and approval of the following by the primary RAC reviewers: (1) Intra-pleural administration of the adenovirus vector will be eliminated from the protocol; therefore, a revised protocol and Informed Consent document are required; and (2) The protocol will be revised to include patient sputum titration assays of the adenovirus on 293 cells (for both the wildtype and vector adenoviruses) to be conducted until virus is no longer detectable (patients will be isolated for a period of 1 week).

The RAC deferred the intrapleural administration portion of the protocol until the investigator returns to the full RAC with a revised protocol that includes toxicity data demonstrating the effect of the adenovirus vector and cisplatin in an appropriate animal model. The RAC *strongly* recommended the cotton rat as the most appropriate model.

XVI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *INJECTION OF GLIOBLASTOMA PATIENTS WITH TUMOR CELLS GENETICALLY MODIFIED TO SECRETE INTERLEUKIN-2 (IL-2): A PHASE I STUDY*/DRS. SOBOL AND ROYSTON

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Robert Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California. This protocol is a resubmission of a protocol that was disapproved by the RAC at its December 2-3, 1993, meeting. The majority of the RAC members concluded that the preclinical data derived from a single patient protocol was inadequate to justify the proposal.

This revised protocol involves subcutaneous injection of irradiated tumor cells secreting IL-2 in an attempt to stimulate antitumor immunity in patients with glioblastoma. Both autologous and allogeneic human leukocyte antigen A2 (HLA-A2) positive tumor cells

will be used. Preclinical animal data and data from the single patient entered on the previous study indicate the possible efficacy of this approach. Since their previous submission, the investigators have documented the dose of irradiation required for complete tumor cell killing and that this dose allows continued IL-2 production for at least 2 weeks. The protocol is well written and has been approved by both the local IRB and the IBC. Potential danger to patients or other individuals from a recombinant DNA standpoint is low. Dr. Miller recommended approval of the protocol.

Review--Dr. Straus (presented by Dr. Wivel)

Dr. Wivel summarized Dr. Straus' written comments. Dr. Straus stated that the protocol is not fundamentally changed from the prior submission. This resubmission addresses most of the concerns raised by the RAC members during the previous review. However, the investigators have failed to address the issue of the necessity for the two study arms, i.e., autologous and allogeneic tumor cells. The use of allogeneic cells is not scientifically validated; therefore, the investigators have agreed to delete the allogeneic arm of the study in their written response to the primary review. Dr. Straus recommended approval of the protocol based on deletion of the allogeneic tumor cell arm of the study.

Review--Ms. Meyers (presented by Dr. Walters)

Dr. Walters summarized the written comments submitted by Ms. Meyers. Ms. Meyers questioned the use of a cell line derived from the deceased glioblastoma patient. However, the investigators have since agreed to delete the allogeneic tumor cell arm of the study. Ms. Meyers expressed concern about the use of immunotherapy for the treatment of brain tumors. The assumption that intracranial tumors can be cured by immunotherapy is based on an article published in 1983. Adoptive immunotherapy has not led to any major therapeutic breakthroughs for cancer treatment.

Investigator Response--Dr. Sobol

Dr. Sobol stated that based on Dr. Straus' and Ms. Meyers' comments, the trial will only involve autologous cells. IL-2 transduced cells will be compared to nontransduced cells. Dr. Sobol agreed to the reviewer's suggestion that both transduced and nontransduced cells should receive identical doses of irradiation. The highest dose of irradiation will be used that does not interfere with IL-2 production.

Committee Motion

The RAC approved a motion made by Dr. Miller and seconded by Dr. Post to accept the protocol (with deletion of the allogeneic cell study arm) submitted by Drs. Robert Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, by a vote of 13 in favor, 0 opposed, and no abstentions.

XVII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN

GENE TRANSFER PROTOCOL ENTITLED: *IL-12 GENE THERAPY USING DIRECT INJECTION OF TUMOR WITH GENETICALLY ENGINEERED AUTOLOGOUS FIBROBLASTS*/**DR. LOTZE**

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Miller noted that the investigator did not meet the deadline for submission of data; therefore, the review process has been impeded. At the time of submission, the entire vector sequence was not included, the vector was misnamed, preclinical data on IL-12 production by transduced cells was submitted late, and the Southern blot data was incomplete. Discussion ensued regarding the appropriateness of reviewing this protocol given the timely delay in submission of relevant data. A decision was made to review the protocol despite these delays. The RAC members noted their dissatisfaction with the manner in which the investigator handled the submission.

Subjects will receive intratumoral injections of irradiated autologous fibroblasts that have been transduced with the gene for IL-12. The investigator hypothesized that local production of IL-12 may promote tumor destruction. The RAC has previously recommended approval of studies involving other cytokine genes with this strategy. Dr. Miller raised several issues: (1) Is the retroviral vector construct, TFG-IL-12-neo, stable and suitable for use in humans? Since IL-12 has 2 subunits, the proposed vector expresses both subunits and neo^R which will be used as a selectable marker. Expression of these 3 genes is accomplished using 2 identical ribosome entry sites (IRES) that allow translation of all 3 gene products from a single mRNA species. The feasibility of this approach should be documented, i.e., adequate levels of IL-12 production and lack of rearrangement during transduction. Southern blot analysis of vector DNA from transduced cells should be provided to demonstrate the lack of rearrangement. (2) Why did the investigators propose a different vector for the human study than was used for the preclinical experiments. (3) What is the rationale for initiating the IL-12 trial when the results of the IL-4 trial have not been submitted? (4) The animal data indicates that only intermediate doses of IL-12 are effective in tumor reduction, high doses of IL-12 have little antitumor effect. Does the IL-12 dose have to be carefully titrated in humans to obtain a response? (5) IL-12 toxicity studies have not been submitted for human subjects or large primates; therefore, can possible adverse effects to humans be predicted for the proposed doses of IL-12?

Review--Dr. Parkman

Dr. Parkman stated that there are 3 critical issues that should be addressed for any protocol: (1) the vector, (2) the preclinical data, and (3) the toxicity data.

In regard to preclinical studies, data was submitted only for a preimmunization animal model. Animals were preimmunized with IL-12 transduced cells. Data was not submitted from a preexisting tumor model until yesterday. These data demonstrated IL-

12 transduced cells resulted in regression of contralateral tumors. Systemic IL-12 toxicity data demonstrated tumor regression 28 days following inoculation of the primary tumors. The proposed starting dose for the human protocol is 10 ng IL-12/24 hours. This dose is 1/70th the dose used for the murine systemic administration studies. In primates, the maximum tolerated dose of IL-12 is as high as 1 mg/kg body weight. Dr. Parkman said the safety margin represented by these recent studies is more than adequate. However, the RAC's concerns about the nature of the vector remain to be resolved.

Review--Ms. Meyers (presented by Dr. Walters)

Dr. Walters summarized the written comments submitted by Ms. Meyers. Ms. Meyers noted that the types of cancer proposed for treatment and the number of patients are not clearly stated in the protocol. Will the maximum number of subjects entered on the study be 18 or 43? Ms. Meyers suggested that Informed Consent issues, i.e., costs for non-negligent injuries, alternative therapies, life-long follow-up, contraception, and autopsy should be clearly described.

Other Comments

Dr. Parkman inquired about the exact vector that will be used for the human study. He agreed with Dr. Miller's comments that the issue of vector rearrangement should be addressed. Dr. Chase inquired about the rationale for testing a broad range of interleukin cytokines. Does the adoptive transfer of cytokine-producing cells hold promise for the treatment of cancer? Discussion ensued about the use of cytokines for the treatment of cancer. The consensus of the RAC was that this area of research may be beneficial based on preclinical data; therefore, human studies are of value.

Dr. Zallen agreed with the comments submitted by Ms. Meyers noting that the description of the murine virus should be revised such that it is more understandable to laypersons.

Investigator Response--Dr. Lotze

Dr. Lotze explained that the terms TFG-IL-12 and TFG-IL-12-Neo are used interchangeably. The vector is derived from the MFG vector that was made by Dr. Richard Mulligan of the Whitehead Institute, Cambridge, Massachusetts. MFG was designed for a single gene insert, whereas, DFG is for a double gene insert. The vector construct encodes the genes for the p35 and p40 subunits of IL-12. TFG refers to the DFG construct with the addition of *neo*^{R}.

With regard to Dr. Miller's question regarding the vector structure, Dr. Lotze explained that *neo*^{R} was placed between the *p35* and *p40* subunit genes to prevent splicing out of *neo*^{R} following selection of transduced cells in G418. Southern blot analysis confirms the structure of the vector DNA in the transduced cells. This construct has two identical 600 base pair IRES elements for internal initiation of protein synthesis. There was concern that homologous recombination of these elements would splice out the middle portion of

the insert sequences; therefore, *neo*^{R} was inserted between these 2 elements to prevent splicing. Autologous fibroblasts will be transduced with the TFG vector, selected in G418, tested for IL-12 production, and injected into the patient. Southern blot analysis of a fibroblast cell line transduced with TFG demonstrated the appropriate 4 kb band, not a 2 kb band. These data indicate that there is no evidence of a splice event.

Dr. Lotze explained that the IL-12 cytokine was chosen based on promising preclinical data. Data suggests that IL-12 is involved in early immune response and rejection of subcutaneous tumors. The interleukins are part of a complex immunological process in which significant knowledge is being gained. He hypothesized that positive results from both the IL-12 and IL-4 human trials could lead to a combination therapy of these 2 cytokines. IL-12 has been extensively tested in primates and has been demonstrated effective at doses much lower than those that are toxic, many orders of magnitude below toxic levels observed in primates.

The *lacZ* mobilization assay has been used to determine the presence of helper virus. Helper virus has not been detected in a background of 1 x 10^6 vector particles. Dr. Lotze assured Dr. Miller that the supernatant from transduced autologous fibroblasts will be assayed for helper virus prior to administration to the patient. Vector production and testing will be performed at the University of Pittsburgh.

Dr. Lotze summarized the preliminary results of his ongoing IL-4 protocol. A total of 9 patients have been treated, 5 with melanoma, 1 with renal cell carcinoma, and 3 with colorectal carcinoma. Between 80,000 and 100,000 units of IL-4 are expressed by cells injected at the primary and secondary vaccine sites. Tumor specific T cell responses, increased numbers of infiltrating T lymphocytes, expression of vascular cell adhesion molecules (VCAM) by endothelial cells, and migration of infiltrating lymphocytes out of the vessels has been observed in response to this treatment. These responses are consistent with the proinflammatory effects of IL-4 observed in preclinical experiments. Early expression of IL-4 gene has been demonstrated by RT/PCR assays. These assays demonstrate that 7 days after vaccination, the IL-4 copy number per ng total RNA is substantially reduced in biopsies taken at the injection site. Only the IL-4 producing vaccine sites are associated with T cell growth. Patients entered on the IL-4 vaccine protocol have evidence of specific T cell proliferation in their tumors. Another 11 patients will be entered on this protocol prior to completion of the study.

Given the unique aspects of IL-12 and IL-4 biology, these 2 cytokines should be tested separately. The major difference between the IL-12 and IL-4 studies is that the IL-12 fibroblasts will be injected directly into the tumor. The IL-4 protocol involves subcutaneous injection of transduced cells into the subject's back. The proposed IL-12 trial is a natural extension of the IL-4 study.

Dr. Lotze presented additional data demonstrating tumor regression in response to systemic IL-12 administration in mice. Dr. Miller noted that the vector construct used for the murine studies is different from the vector proposed for the human study. The murine vector has the *neo*^{R} gene at the end of the *p40* and *p35* subunit genes rather than

between these genes. Dr. Miller expressed his dissatisfaction with the Southern blot data provided in response to his concerns about vector rearrangement. Drs. Saha and Post suggested that additional data should be requested as a stipulation for approval. Dr. Miller stressed that the vector construct to be administered to the patients should be adequately characterized. There were several statements that failure of the investigators to comply with the RAC timetable for submission of data made the approval questionable. Dr. Parkman suggested that the *NIH Guidelines* should be amended such that data submitted less than 2 weeks before the meeting at which the protocol will be reviewed should not be permitted. Ms. Wilson explained that such a provision is included in Appendix M-III-B-3 of the *NIH Guidelines*. Several RAC members stated that it is RAC's obligation to proceed with the review of the late data. The consensus of the RAC was that the protocol should be reviewed since it was on the table.

Committee Motion

The RAC approved a motion made by Dr. Post and seconded by Dr. Motulsky to approve the protocol submitted by Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, by a vote of 10 in favor, 0 opposed, and 3 abstentions. Approval of the protocol is contingent on the review and approval of the following by Drs. Miller and Parkman: (1) complete vector sequence, including a detailed restriction enzyme digestion map relevant to the vector backbone; and (2) Southern blot analysis of cells transduced with the vector constructs with and without the gene inserts, demonstrating concordance with the restriction enzyme analysis and sequence data.

XVIII. CONTINUED DISCUSSION OF THE AMENDMENT TO PART I-D, INFORMED CONSENT, OF THE *POINTS TO CONSIDER* OF THE *NIH GUIDELINES*/DR. ZALLEN

Dr. Walters stated that the RAC's previous comments and suggestions on the draft revision of Part I-D, Informed Consent, of the *NIH Guidelines*, have been incorporated into a revised document. Under the section on *Reproductive Considerations*, the statement about pregnant and lactating women has been moved from last to the first sentence. Dr. Motulsky suggested that this section be revised to include male contraception. Dr. Smith suggested that the first sentence should become the last sentence for clarity. Dr. Smith suggested that Section I-D-2-a-(5) should be revised to clarify the use of descriptors. The consensus of the RAC was that the revised language will read:

> "If verbal descriptors (e.g., *rare*, *uncommon*, or *frequent*) are used to express quantitative information regarding risk, these terms should be explained."

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Miller to accept minor editorial changes to Part I-D, Informed Consent, by a vote of 13 in favor, 0 opposed, and no abstentions.

The amended version of Part I-D, Informed Consent, of the *Points to Consider* of the *NIH Guidelines* reads:

"Part I-D. Informed Consent"

"In accordance with the requirements of DHHS regulations for the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should also indicate how the Informed Consent document makes clear the special requirements of gene transfer research."

"Part I-D-1. Communication About the Study to Potential Participants"

"Part I-D-1-a. Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?"

"Part I-D-1-b. How will the major points covered in Parts I-A through I-C be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?"

"Part I-D-1-c. What is the length of time that potential participants will have to make a decision about their participation in the study?"

"Part I-D-1-d. If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?"

"Part I-D-2. Informed Consent Document"

"Investigators submitting human gene transfer proposals for Recombinant DNA Advisory Committee review must include the Informed Consent document as approved by the local Institutional Review Boards. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a "marker" or to enhance the power of immunotherapy for cancer."

"Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent process should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document, or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are

omitted from the consent process or the Informed Consent document."

"Part I-D-2-a. General Requirements of Human Subjects Research"

"Part I-D-2-a-(1). Description/Purpose of the Study"

"The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component."

"Part I-D-2-a-(2). Alternatives"

"The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches."

"Part I-D-2-a-(3). Voluntary Participation"

"The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled."

"Part I-D-2-a-(4). Benefits"

"The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others."

"Part I-D-2-a-(5). Possible Risks, Discomforts, and Side Effects"

"There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization."

"If verbal descriptors (e.g., "rare", "uncommon", or "frequent") are used to express quantitative information regarding risk, these terms should be explained."

"The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials

previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe."

"The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started."

"Part I-D-2-a-(6). Costs"

"The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved."

"Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury."

"Part I-D-2-c. Specific Requirements of Gene Transfer Research"

"Part I-D-2-c-(1). Reproductive Considerations"

"To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified."

"The inclusion of pregnant or lactating women should be addressed."

"Part I-D-2-c-(2). Long-Term Follow-Up"

"To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The principal investigator should request that subjects continue to provide a current address and telephone number."

"The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed."

"Part I-D-2-c-(3). Request for Autopsy"

"To obtain vital information about the safety and efficacy of gene transfer, autopsies are to be performed, if feasible. Subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance."

"Part I-D-2-c-(4). Interest of the Media and Others in the Research"

"To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (1) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (2) that representatives of applicable Federal agencies (e.g., the NIH and the FDA), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records."

XIX. FUTURE MEETINGS OF THE RAC

The next meeting of the RAC will be September 12-13, 1994, at the NIH, Building 31C, Conference Room 6, Bethesda, Maryland.

XX. ADJOURNMENT

Dr. Walters thanked Dr. Post for extending his service on the RAC to this meeting. He adjourned this meeting of the RAC at 1:00 p.m. on June 10, 1994.

Nelson A. Wivel

Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: 9/12/94

LeRoy B. Walters

LeRoy B. Walters, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health

RECOMBINANT DNA ADVISORY COMMITTEE

CHAIR

WALTERS, LeRoy B., Ph.D. (95)
Director, Center for Bioethics
Kennedy Institute of Ethics
Georgetown University
1437 37th St., N.W., Room 215
Washington, D.C. 20057
202 687-6771

ANDREWS, Lori B., J.D. (97)
Professor of Law
Chicago-Kent College of Law
565 W. Adams Street
Chicago, Illinois 60661
312 906-5359

BRINCKERHOFF, Constance E., Ph.D. (94)
Professor, Dept. of Medicine
Dartmouth Medical School
2 Maynard Street
Hanover, New Hampshire 03755
603 650-1609

BUC, Nancy L., LL.B. (94)
Partner
Buc, Levitt, & Beardsley
1800 M Street, N.W., Suite 710 S.
Washington, D.C. 20036
202 736-3610

CAPRON, Alexander M., LL.B. (94)
Professor
The Law Center
University of Southern California
Los Angeles, California 90089-0071
213 740-2557

CHASE, Gary A., Ph.D. (95)
Professor of Medicine
Georgetown University Medical Center
Medical/Dental Building, NW103
3900 Reservoir Road
Washington, D.C. 20007
202 687-7007

DeLEON, Patricia A., Ph.D. (95)
Professor, Dept. of Human Genetics
School of Life and Health Sciences
University of Delaware, 219 McKinly
Newark, Delaware 19716
302 831-2249

DOI, Roy H., Ph.D. (94)
Professor, Section of Mol. & Cell. Biology
Division of Biological Sciences
University of California
Davis, California 95616
916 752-3191

DRONAMRAJU, Krishna R., Ph.D. (95)
President
Foundation for Genetic Research
P.O. Box 27701-497
Houston, Texas 77227
713 961-0415

ERICKSON, Robert P., M.D. (96)
Professor, Dept. of Pediatrics, Molecular & Cellular Biology
University of Arizona, Room 4341A
Tucson, Arizona 85724
602 626-5483

GINSBURG, David, M.D. (97)
Professor, Dept. of Internal Med. & Human Genetics, MSRB1, Room 4520
Howard Hughes Medical Institute
University of Michigan
1150 W. Medical Center Drive
Ann Arbor, Michigan 48109-0650
313 747-4808

HASELKORN, Robert, Ph.D. (94)
Professor
Department of Molecular Genetics & Cell Biology
University of Chicago, 920 E. 58th St.
Chicago, Illinois 60637
312 702-1069 or 1620

MEYERS, Abbey S. (96)
Executive Director
National Organization for Rare Disorders
P.O. Box 8923
New Fairfield, Connecticut 06812
203 746-6518

MILLER, A. Dusty, Ph.D. (95)
Associate Member
Program in Molecular Medicine
Fred Hutchinson Cancer Research Center
1124 Columbia Street
Seattle, Washington 98104
206 667-2890

MOTULSKY, Arno G., M.D. (96)
Professor
Department of Medicine & Genetics
University of Washington Medical School
RG-25
Seattle, Washington 98195
206 543-3593

PARKMAN, Robertson, M.D. (95)
Head, Division of Res. Immunology--Bone Marrow Transplantation
Childrens Hospital of Los Angeles
4650 Sunset Boulevard
Los Angeles, California 90027
213 669-2546

POST, Leonard E., Ph.D. (93)
Vice President, Experimental Therapy
Parke-Davis Pharmaceutical Division
2800 Plymouth Road
Ann Arbor, MI 48105
313 996-2899

ROSS, Gail S., Ph.D. (97)
Research Director, High-risk Infant Follow-up Program, Room N-506
New York Hospital, Perinatology Center
525 E. 68th Street
New York, New York 10021
212 746-3548

SAHA, Bratin K., Ph.D. (97)
Asst. Professor, Dept. of Pathology
Winship Cancer Center, Room 5223
Emory University, 1327 Clifton Road, N.E.
Atlanta, Georgia 30322
404 248-4432

SAMULSKI, Richard J., Ph.D. (97)
Director, Gene Therapy Center
G44 Wilson Hall, CB# 7352
University of North Carolina
Chapel Hill, North Carolina 27599-7352
919 962-1224

SECUNDY, Marian G., Ph.D. (96)
Professor, Dept. of Community Health & Family Practice
Howard University College of Medicine
520 W Street, N.W.
Washington, D.C. 20059
202 806-6397

SMITH, Brian R., M.D. (96)
Associate Professor of Laboratory Medicine
Internal Medicine and Pediatrics
Yale University School of Medicine
333 Cedar Street
New Haven, Connecticut 06510-3333
203 785-2286

STRAUS, Stephen E., M.D. (96)
Chief
Laboratory of Clinical Investigation, NIAID
National Institutes of Health
Building 10, Room 11N228
Bethesda, Maryland 20892
301 496-5221

ZALLEN, Doris T., Ph.D.
Associate Professor
Science Studies and Humanities
VA Polytechnic Institute & State Univ.
Humanities Center, Lane Hall-354
Blacksburg, Virginia 24061
703 231-4216

EXECUTIVE SECRETARY

WIVEL, Nelson A., M.D.
Director
Office of Recombinant DNA Activities
National Institutes of Health, 31/4B11
Bethesda, Maryland 20892
301 496-9838

AD HOC CONSULTANT

GINSBERG, Harold, M.D.
Senior Scientist
NIAID, Twinbrook II, Lab. #87
National Institutes of Health
12441 Parklawn Drive
Rockville, Maryland 20852
301 496-2976

NON-VOTING AGENCY REPRESENTATIVES

DEPARTMENT OF AGRICULTURE (USDA)

TOLIN, Sue A., Ph.D.
Science and Education
Cooperative State Research Service
USDA, Suite 330, Aerospace Bldg.
Washington, D.C. 20250-0220
202 447-5741

GAY, Cyril G., Ph.D. (ALT)
Veterinary Biologics
USDA, Federal Building, Room 836
6505 Belcrest Road
Hyattsville, MD 20782
301 436-8674

DEPARTMENT OF COMMERCE

GREIFER, Bernard, Ph.D.
Economics & Statistics Administration
Office of Policy Analysis
Room H-4858
Department of Commerce
Washington, D.C. 20230
202 482-3078

SHYKIND, Edwin, Ph.D. (ALT)
Trade Development, Room H-4045
International Trade Administration
Department of Commerce
Washington, D.C. 20230
202 377-4694

DEPARTMENT OF ENERGY

DRELL, Daniel W., Ph.D.
Office of Health and Environmental Research
Department of Energy, ER-72
Washington, D.C. 20545
301 903-4742

DEPARTMENT OF HEALTH AND HUMAN SERVICES (DHHS)

DHHS Alcohol, Drug Abuse and Mental Health Administration

GAUSE, Emily M.
Office of the Science Advisor
Alcohol, Drug Abuse and Mental Health Administration
5600 Fishers Lane, Room 1295
Rockville, Maryland 20857
301 443-4335

AUTRY, Joseph H., III, M.D. (ALT)
Office of Policy Coordination
Alcohol, Drug Abuse and Mental Health Administration
5600 Fishers Lane, Room 12C-06
Rockville, Maryland 20857
301 443-4111

DHHS Centers for Disease Control and Prevention (CDCP)

GUINAN, Mary E., M.D., Ph.D.
Office of the Director
Centers for Disease Control and Prevention
1600 Clifton Road, D22
Atlanta, Georgia 30333
404 639-3701

DHHS, CDC National Institute for Occupational Safety and Health

LEMEN, Richard A.
National Institute for Occupational
Safety and Health
4676 Columbia Parkway
Cincinnati, Ohio 45226
513 684-8302

DHHS Food and Drug Administration

NOGUCHI, Philip, M.D.
Division of Cellular and Gene Therapies
Center for Biologics Evaluation & Res.
FDA, HFM 515, Bldg. 29, Room B24
1401 Rockville Pike
Rockville, Maryland 20852-1448
301 496-4709

KUTA, Alexander, Ph.D. (ALT)
Office of Therapeutics, Research & Review
Center for Biologics Evaluation & Res.
FDA, HFM-99, Room 200N
1401 Rockville Pike
Rockville, Maryland 20852-1448
301 594-5114

DHHS Health Resources and Services Administration

MANLEY, Audrey, M.D.
Division of Health Service Corps
Administration
Health Resources & Services Administration
5600 Fishers Lane, Room 7A39
Rockville, Maryland 20857
301 443-2900

DHHS Office of the Assistant Secretary for Health

HUDSON, Kathy
Office of Health Planning and Evaluation
DHHS Office of the Assistant Secretary
for Health
200 Independence Avenue, S.W., Room 740G
Washington, D.C. 20201
202 690-8033

DEPARTMENT OF THE INTERIOR

PIMENTEL, Mariano B., M.D.
Division of Medical & Health Services
Department of the Interior, Room 7045
18th & C Street, N.W.
Washington, D.C. 20240
202 343-2081

DEPARTMENT OF LABOR

YODAIKEN, Ralph E., M.D.
Senior Medical Advisor
USDOL/OSHA, Room N-3653
200 Constitution Avenue, N.W.
Washington, D.C. 20210
202 523-7047

DEPARTMENT OF STATE

SAVAGE, Eleanor W.
Office of Ecology, Health & Conservation
OES/EHS, Room 4325
Department of State
2201 C Street, N.W.
Washington, D.C. 20520
202 647-2418

WEST, Peter A., M.D. (ALT)
Office of Ecology, Health & Conservation
OES/EHC, Room 4325
Department of State
2201 C Street, N.W.
Washington, D.C. 20520
202 647-2418

DEPARTMENT OF TRANSPORTATION

CUSHMAC, George E., Ph.D.
Research & Special Program Administration
Department of Transportation
400 7th Street, S.W.
Washington, D.C. 20590
202 426-2311

ENVIRONMENTAL PROTECTION AGENCY

FREDERICK, Robert J., Ph.D.
Office of Environmental Processes
and Effects Research, RD-682
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
202 382-5989

MILEWSKI, Elizabeth, Ph.D. (ALT)
Office of Pesticides & Toxic Substances
Environmental Protection Agency, RD-682
401 M Street, S.W.
Washington, D.C. 20460
202 382-2914

NATIONAL SCIENCE FOUNDATION

HARRIMAN, Philip, Ph.D.
Division of Molecular Biosciences
National Science Foundation, Room 325
4201 Wilson Boulevard
Arlington, VA 22230
703 306-1441

LIAISON REPRESENTATIVES

TAKAGI, Yasuyuki, M.D.
Division of Molecular Genetics
Fujita Gakuen Health University
Toyoake, Aichi, 470-11
JAPAN
(0562) 93-9375

JONES, Daniel P., Ph.D.
Humanities, Science and Technology
Division of Research Programs
National Endowment for the Humanities
Washington, D.C. 20506
202 786-0207

FAYL, Gilbert
Counselor, Science & Technology Section
Delegation of the Commission of the
European Communities
2100 M Street, N.W., 7th Floor
Washington, D.C. 20037
202 862-9575

HUMAN GENE TRANSFER PROTOCOLS

8810-001[a] (M) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2 and Tumor Infiltrating Lymphocytes.*
RAC Approval: 10-3-88/NIH Approval: 3-2-89
Minor Modification: 3-30-90

9007-002[a,b] (T) Blaese, R. Michael, National Institutes of Health, Bethesda, Maryland; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with Autologous Lymphocytes Transduced with the Human ADA Gene: An Experimental Study*
RAC Approval: 7-31-90/NIH Approval: 9-6-90
Major Amendment/RAC Approval: 2-10-92
Major Amendment/NIH Approval: 4-22-92
Minor Modification: 4-21-93
Minor Modification: 6-7-93

9007-003[a] (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor.*
RAC Approval: 7-31-90/NIH Approval: 9-6-90
Minor Modification: 2-17-94

9102-004[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Hurwitz, Craig; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 2-4-91/NIH Approval: 7-12-91
Minor Modification: 11-91
CLOSED: 1-15-93

9105-005[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91
CLOSED: 6-1-92

9105-006[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91
CLOSED: 4-9-93

9105-007[a] (M) Deisseroth, Albert B., M.D. Anderson Cancer Research Center, Houston, Texas; *Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 4-19-93
CLOSED: 6-1-93

9105-008 (M) Ledley, Fred D.; Woo, Savio; Ferry, George; and Hartwell, Whigennand, Baylor College of Medicine, Houston, Texas; *Hepatocellular Transplantation in Acute Hepatic Failure and Targeting Genetic Markers to Hepatic Cells.*
RAC Approval: 5-30-91/NIH Approval: 7-12-91
CLOSED: Protocol Never Initiated

9105-009[a] (M) Lotze, Michael T., University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; *The Administration of Interleukin-2 and Tumor Infiltrating Lymphocytes to Patients with Melanoma.*
RAC Approval: 5-30-91/NIH Approval: 1-17-92
Minor Modification: 11-30-92

9110-010[a] (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91
Minor Modification: 7-9-92

9110-011 (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91

9110-012[a] (T) Wilson, James M., University of Pennsylvania Medical Cenyrt, Philadelphia, Pennsylvania; *Ex Vivo Gene Therapy of Familial Hypercholesterolemia.*
RAC Approval: 10-8-91/NIH Approval: 11-14-91
Minor Modification: 12-3-92
Minor Modification: 8-18-93

9202-013[a] (T) Nabel, Gary J., University of Michigan, Ann Arbor, Michigan; *Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors.*
RAC Approval: 2-10-92/NIH Approval: 4-17-92
Minor Modification: 1-22-93
Minor Modification: 1-93
CLOSED: 11-19-92

9202-014 (M) Cornetta, Kenneth, Indiana University, Indianapolis, Indiana; *Retroviral-Mediated Gene Transfer of Bone Marrow Cells during Autologous Bone Marrow Transplantation for Acute Leukemia.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-015 (M) Economou, James S. and Belldegrun, Arie, University of California at Los Angeles, Los Angeles, California; *The Treatment of Patients with Metastatic Melanoma and Renal Cell Cancer Using In Vitro Expanded and Genetically-Engineered (Neomycin Phosphotransferase) Bulk, CD8 (+) and/or CD4(+) Tumor Infiltrating Lymphocytes and Bulk, CD8(+) and/or CD4(+) Peripheral Blood Leukocytes in Combination with Recombinant Interleukin-2 Alone, or with Recombinant Interleukin-2 and Recombinant Alpha Interferon.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-016[a] (T) Freeman, Scott M., Tulane University Medical Center, New Orleans, Louisiana; *Gene Transfer for the Treatment of Cancer.*
RAC Approval: 2-10-92/NIH Approval: 2-5-93
Minor Modification: 8-6-93

9202-017[b] (M) Greenberg, Philip D. and Riddell, Stanley, Fred Hutchinson Cancer Research Center, University of Washington, Seattle; *Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Genetically Modified CD8+ HIV-Specific T Cells in HIV Seropositive Individuals.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92
Major Amendment/RAC Approval: 9-9-93
Major Amendment/NIH Approval: 10-7-93

9206-018 (T) Brenner, Malcolm K.; Furman, Wayne; Santana, Victor; Bowman, Laura; and Meyer, William, St. Jude Children's Research Hospital, Memphis, Tennessee; *Phase I Study of Cytokine-Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92

9206-019[a] (T) Oldfield, Edward, National Institutes of Health, Bethesda, Maryland; *Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92
Minor Modification: 11-29-93

9206-020[a] (M) Deisseroth, Albert B., MD Anderson Cancer Center, Houston, Texas; *Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 4-19-93

9206-021 (T) Gansbacher, Bernd; Houghton, Alan; and Livingston, Philip, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with HLA-A2 matched Allogeneic Melanoma Cells that Secrete Interleukin-2 in Patients with Metastatic Melanoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9206-022[a] (T) Gansbacher, Bernd; Motzer, Robert; Houghton, Alan; and Bander, Neil, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 3-31-93

9206-023[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Multiple Myeloma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94
Minor ModificationL 3-23-94

9206-024* (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Breast Cancer.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94

9206-025* (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 1-6-94
Minor Modification: 3-23-94

9209-026 (M) Walker, Robert E., National Institutes of Health, Bethesda, Maryland; *A Study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in HIV Infected Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 9-3-93

9209-027* (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Phase I/II Study of PIXY321, a rhGM-CSF/rhIL-3 Fusion Protein or rhG-CSF, for the Mobilization of Peripheral Blood Stem Cells for Autologous Stem Cell Transplantation.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93
Minor Modification: 2-25-94

9209-028* (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *Evaluation of th Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93
Minor Modification 2-25-94
CLOSED: 2-25-94 (Merged with protocol # 9209-027)

9209-029 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Trial of G-CS Stimulated Peripheral Blood Stem Cells for Engraftment in Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93
CLOSED: Protocol Never Initiated

9209-030 (M) Deisseroth, Albert B., University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients.*
RAC Approval: 9-14-92/NIH Approval: 12-2-93
Minor Modification: 11-3-93
Minor Modification: 11-18-93

9403-031/9209-031 (T) Roth, Jack A., The University of Texas MD Anderson Cancer Center, Houston, Texas; *Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer NSCLC).*
9209-031/ RAC Approval of Original Protocol: 9-15-92/RAC Approval of Original Protocol Withdrawn 12-3-93
9403-031/ RAC Approval of Resubmitted Protocol: 3-4-94/NIH Approval of Resubmitted Protocol: Pending

9209-032 (M) Brenner, Malcolm K., St. Jude Children's Research Hospital, Memphis, Tennessee; A *Phase II Trial of the Baxter Neuroblastoma Bone Marrow Purging System Using Gene Marking to Assess Efficacy.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9209-033 (T) Lotze, Michael T. and Rubin, Joshua T., University of Pittsburgh, Pittsburgh, Pennsylvania; *Gene Therapy of Cancer: A Pilot Study of IL-4 Gene Modified Antitumor Vaccines.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9212-034* (T) Crystal, Ronald G., New York Hospital-Cornell Medical Center; *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.*
RAC Approval: 12-3-92/NIH Approval: 4-16-93
Minor Modification: 5-17-93
Minor Modification: 10-8-93
Minor Modification: 11-29-93
Minor Modification: 2-4-94

9212-035* (T) Wilson, James M., University of Pennsylvania Medical Center, Philadelphia, Pennsylvania; *Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial.*
RAC Approval: 12-3-92/NIH Approval: 8-26-93
Minor Modification: 8-17-93
Minor Modification: 8-26-93

9212-036[a] (T) Welsh, Michael J., Howard Hughes Medical Institute, Iowa City, Iowa, and Smith, Alan E., Genzyme Corporation, Framingham, Massachusetts; *Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium.*
RAC Approval: 12-4-92/NIH Approval: 4-16-93
Minor Modification: 10-18-93

9303-037 (T) Culver, Kenneth W., Iowa Methodist Medical Center, Des Moines, Iowa, and Van Gilder, John C.; University of Iowa, Iowa City, Iowa; *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.*
RAC Approval: 3-1-93/NIH Approval: 4-16-93

9303-038 (M) Heslop, Helen E.; Brenner, Malcolm K.; and Rooney, Cliona, St. Jude Children's Research Hospital, Memphis, Tennessee; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-039 (M) Brenner, Malcolm K.; Krance, Robert; Heslop, Helen E.; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital Memphis, Tennessee; *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-040 (T) Simons, Jonathan, Johns Hopkins Oncology Center, Baltimore, Maryland; *Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.*
RAC Approval: 3-1-93/NIH Approval: 12-2-93

9303-041 (T) Wilmott, Robert W. and Whitsett, Jeffrey, Children's Hospital Medical Center, Cincinnati, Ohio, and Trapnell, Bruce; Genetic Therapy, Inc., Gaithersburg, Maryland; *A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-042[a] (T) Boucher, Richard C. and Knowles, Michael R., University of North Carolina, Chapel Hill, North Carolina; *Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity.*
RAC Approval: 3-2-93/NIH Approval: 10-7-93
Minor Modification: 2-17-94

9306-043 (T) Seigler, Hilliard F., Duke University Medical Center, Durham, North Carolina; *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-044[a] (T) Deisseroth, Albert B.; Kavanagh, John; and Champlin, Richard, University of Texas MD Anderson Cancer Center, Houston, Tex *Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.*
RAC Approval: 6-7-93/NIH Approval: 12-2-93
Minor Modification: 2-25-94
Minor Modification: 2-25-94

9306-045 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-046 (T) Barranger, John A., University of Pittsburgh, Pittsburgh, Pennsylvania, *Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34(+) Cells.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-047 (T) Karlsson, Stefan and Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland, and Kohn, Donald B., Childrens Hosp Los Angeles, Los Angeles, California; *Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-048 (T) Galpin, Jeffrey E., University of Southern California, and Casciato, Dennis A., Shared Medical Research Foundation, Tarzana, California; *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-049 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *A Molecular Genetic Intervention for AIDS - Effects a Transdominant Negative Form of Rev.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-EXP (T) Sobol, Robert and Royston, Ivor, San Diego Regional Cancer Center, San Diego, California; *CONFIDENTIAL.*
NIH Approval: 12-28-92
Major Amendment (Expedited Review)/NIH Approval: 5-11-93
CLOSED: 10-20-93 (Patient death)

9306-050 (T) Raffel, Corey, Childrens Hospital Los Angeles, Los Angeles, California, and Culver, Kenneth, Iowa Methodist Medical Center, Des Moines, Iowa; *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-051 (T) Hesdorffer, Charles and Antman, Karen, Columbia University College of Physicians and Surgeons, New York, New York; *Human MDR Gene Transfer in Patients with Advanced Cancer.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-052 (T) Ilan, Joseph, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*
RAC Approval: 6-8-93/NIH Approval: 12-2-93

9309-053[a] (T) Cassileth, Peter; Podack, Eckhard R.; Sridhar, Kasi; University of Miami, and Savaraj, Niramol; Miami Veterans Administration Hospital, Miami, Florida; *Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small Cell Lung Cancer.*
RAC Approval: 9-9-93/NIH Approval: 12-2-93
Minor Modification: 3-23-94

9309-054 (T) O'Shaughnessy, Joyce; National Institutes of Health, Bethesda, Maryland; *Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR-1) into Hematopoietic Stem Cells During Autologous Transplantation after Intensive Chemotherapy for Breast Cancer.*
RAC Approval: 9-9-93/NIH Approval: 10-7-93

9309-055 (T) Kun, Larry E.; Sanford, R. A.; Brenner, Malcolm K.; and Heideman, Richard L.; St. Jude Childrens Research Hospital, Memphis, Tennessee; and Oldfield, Edward H.; National Institutes of Health, Bethesda, Maryland; *Gene Therapy for Recurrent Pediatric Brain Tumors.*
RAC Approval: 9-9-93/NIH Approval: 10-7-93

9309-056 (T) Das Gupta, Tapas K. and Cohen, Edward P.; University of Illinois at Chicago; Chicago, Illinois; *Immunization of Malignant Melanoma Patients with Interleukin 2-Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.*
RAC Approval: 9-10-93/NIH Approval: 4-19-94

9309-057 (T) Wong-Staal, Flossie; Poeschla, Eric; and Looney, David; University of California, San Diego, California; *A Phase I Clinical Trial to Evaluate the Safety and Effects in HIV-1 Infected Humans of Autologous Lymphocytes Transduced with a Ribozyme that Cleaves HIV-1 RNA.*
RAC Approval: 9-10-93/NIH Approval: Pending

9309-058 (T) Economou, James S. and Glasby, John A.; University of California Medical Center, Los Angeles, California; *Genetically Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma.*
RAC Approval: 9-10-93/NIH Approval: 12-2-93

9312-059 (T) Oldfield, Edward H. and Ram, Zvi; NIH, Bethesda, Maryland; *Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis.*
RAC Approval: 12-2-93/NIH Approval: 1-20-94

9312-060 (T) Sobol, Robert E. and Royston, Ivor; San Diego Regional Cancer Center, San Diego, California; *Injection of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2.*
RAC Approval: 12-2-93/NIH Approval: Pending

9312-061 (T) Schuening, Friedrich; Fred Hutchinson Cancer Research Center, Seattle, Washington; *Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.*
RAC Approval: 12-2-93/NIH Approval: Pending

9312-062 (T) Haubrich, Richard; University of California at San Diego Treatment Center, San Diego, California; *An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT(V) (HIV-1 IIIBenv/Retroviral Vector) in HIV-1 Infected Subjects.*
RAC Approval: 12-3-93/NIH Approval: 4-19-94

9312-063 (T) Sznol, Mario; National Institutes of Health, Frederick, Maryland; *A Phase I Trial of B7-Transfected Lethally Irradiated Allogeneic Melanoma Cell Lines to Induce Cell Mediated Immunity Against Tumor-Associated Antigens Presented by HLA-A2 or HLA-A1 in Patients with Stage IV Melanoma.*
RAC Approval: 12-3-93/NIH Approval: 4-19-94

9312-064 (T) Rubin, Joseph, Mayo Clinic, Rochester, Minnesota; *Phase I Study of Immunotherapy of Advanced Colorectal Carcinoma by Direct Gene Transfer into Hepatic Metastases.*
RAC Approval: 12-3-93/NIH Approval: 4-19-94

9312-065 (T) Chang, Alfred E., University of Michigan, Ann Arbor, Michigan; *Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene.*
RAC Approval: 12-3-93/NIH Approval: Pending

9312-066 (T) Sorscher, Eric J. and Logan, James L.; University of Alabama, Birmingham, Alabama; *Gene Therapy for Cystic Fibrosis Using Cationic Liposome Mediated Gene Transfer: A Phase I Trial of Safety and Efficacy in the Nasal Airway.*
RAC Approval: 12-3-93/NIH Approval: Pending

9312-067[a] (T) Welsh, Michael J.; Howard Hughes Medical Institute, Iowa City, Iowa; *Adenovirus-Mediated Gene Transfer of CFTR to the Nasal Epithelium and Maxillary Sinus of Patients with Cystic Fibrosis.*
RAC Approval: 12-3-93/NIH Approval: 2-10-94
Minor Modification: 2-17-94

9403-068 (T) Rosenblatt, Joseph; University of California, Los Angeles, California; and Seeger, Robert; Childrens Hospital, Los Angeles, California; *A Phase I Study of Immunization with Gamma Interferon Transduced Neuroblastoma Cells.*
RAC Approval: 3-3-94/NIH Approval: Pending

9403-069 (T) Walker, Robert; NIH, Bethesda, Maryland; *A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene-Modified Cytotoxic T-Lymphocytes in HIV-Infected Identical Twins.*
RAC Approval: 3-3-94/NIH Approval: Pending

9403-070 (T) Brigham, Kenneth; Vanderbilt University, Nashville, Tennessee; *Expression of an Exogenously Administered Human Alpha-1-Antitrypsin Gene in the Respiratory Tract of Humans.*
RAC Approval: 3-3-94/NIH Approval: Pending

9403-071 (T) Vogelzang, Nicholas; the University of Chicago, Chicago, Illinois; *Phase I Study of Immunotherapy for Metastatic Renal Cell Carcinoma by Direct Gene Transfer into Metastatic Lesions.*
RAC Approval: 3-4-94/NIH Approval: 4-19-94

9403-072 (T) Hersh, Evan; Arizona Cancer Center, Tuscon, Arizona; and Akporiaye; Harris; Stopeck; Unger; and Warneke; the University of Arizona, Tuscon, Arizona; *Phase I Study of Immunotherapy of Malignant Melanoma by Direct Gene Transfer.*
RAC Approval: 3-4-94/NIH Approval: 4-19-94

	T	M	Total (T + M)
RAC APPROVED	50	22	72
NIH DIRECTOR APPROVED	42	22	64[c]

LEGEND:

M = Human Gene Marking Protocol
T = Human Gene Therapy Protocol
[a] = Minor Modification(s)
[b] = Major Amendment(s)
[c] = The number of protocols appearing in the table are greater than the number that appears in the protocol list because of the single *Expedited Review* protocol that was approved by the NIH Director on 12-28-92.
_ = RAC Approved/NIH Approval Pending

May 13, 1994

MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	MINOR MODIFICATION
3/30/90	3/2/89	8810-001	Rosenberg, Steven A.	Lift the cap on the number of patients accrued onto the protocol.
11/91	7/12/91	9102-004	Brenner, Malcolm K., et. al.	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-005	Brenner, Malcolm K., et. al.	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-006	Brenner, Malcolm K., et. al.	Use the retrovirus vector, G1Na, instead of LNL6.
7/9/92	10/15/91	9110-010	Rosenberg, Steven A.	Extend patient eligibility criteria to include patients with advanced breast cancer.
11/30/92	1/17/92	9105-009	Lotze, Michael T.	(1) Eliminate systemic IL-4 therapy, and (2) allow administration of cyclophosphamide prior to TIL infusions.
12/3/92	11/14/91	9110-012	Wilson, James M.	Increase patient accrual from 3 to 5 patients.
1/93	4/17/92	9202-013	Nabel, Gary J.	(1) addition of assays for anti-DNA antibodies on day 28 and week 8; (2) repeat chest and abdominal CTs, when indicated, at 6-8 weeks following initial treatment; (3) additional biopsies performed on days 16 and 20 and as needed to verify gene expression; (4) reduce the duration of the protocol from 16 to 8 weeks; and (5) expand patient eligibility to include those patients who have tolerated previous treatments without side effects at higher doses.
1/22/93	4/17/92	9202-013	Nabel, Gary J.	Deliver HLA-B7 DNA liposome complexes by catheter to pulmonary nodules.
3/31/93	8/14/92	9206-022	Gansbacher, Bernd, et. al.	Use only irradiated SK-RC-39 cell line, not SK-RC-28.
4/19/93	7/12/91	9105-007	Deisseroth, Albert B.	New transduction protocol which allows the use of autologous stroma and growth factors.
4/19/93	8/14/92	9206-020	Deisseroth, Albert B.	Revised transduction procedure that includes the use of autologous stroma and growth factors.
4/21/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treat 2 ADA-deficient newborns (1 at Children's Hospital Los Angeles and 1 at the National Institutes of Health) with ADA gene-corrected autologous hematopoietic stem cells obtained from the patients' umbilical cord and placenta.
5/17/93	4/16/93	9212-034	Crystal, Ronald G.	Decrease the "baseline" and "vehicle control" portions of the protocol from 1 month to 1 week each.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	MINOR MODIFICATION
6/7/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	The investigator is permitted to: (1) modify the patient eligibility criteria to include newborns with ADA deficiency (subjects will receive ADA gene-corrected autologous hematopoietic stem cells obtained from the umbilical cord and placenta), (2) withdraw PEG-ADA at his discretion, and (3) perform bone marrow aspirations as necessary to monitor for gene transduction.
8/6/93	2/5/93	9202-016	Freeman, Scott M.	PI has moved to Tulane University Medical Center. The total number of patients will be divided between Tulane University and the University of Rochester.
8/18/93	8/25/93	9212-035	Wilson, James M.	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/19/93	11/14/91	9110-012	Wilson, James M.	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/26/93	8/26/93	9212-035	Wilson, James M.	(1) the lowest dose of adenovirus vector will be decreased by 100-fold, (2) the highest dose will be decreased by 10-fold, (3) the incremental dose between the patient groups will be reduced from 10-fold to 3-fold, (4) the number of patients within each group will be reduced from three to two, and (5) the total number of patients enrolled will be increased from 12 to 20.
10/8/93	4/16/93	9212-034	Crystal, Ronald, G.	(1) Change in dosing schedule, (2) increase time interval between nasal and lung administration from 1 to 2 days, (3) the time interval between patients within each dosing group will be changed to 2 weeks, (4) the time interval between the dose groups will be changed to 3 weeks, (5) isolation period will be reduced from 10 to 2 days, (6) eliminate requirement for negative pressure isolation of patients, (7) inpatient requirement will be reduced from 2 months to 2 weeks (contingent on 3 consecutive negative cultures), (8) addition of bronchial biopsies, (9) elimination of scintigraphic assessment, (10) elimination of quantitation of sputum volume and sputum culture, (11) patients will enter the 7 day vehicle control period within 2 months of completion of the 7 day baseline period and will receive vector administration within 2 months of completion of the vehicle control period, and (12) the number of chest CT scans will be reduced to 1 baseline scan unless indicated for clinical purposes. Contingency: any patients demonstrating unexpected side effects will receive a CT scan.
10/18/93	4/16/93	9212-036	Welsh, Michael J.	(1) add an additional 3 patients; (2) each additional patient will receive $5x10^7$ IU of the adenovirus vector; (3) the adenovirus vector will be applied to only 1 nostril (saline in the other as a control); (4) the virus treated and saline control tissues will be biopsied (patient #1-day 1, patient #2-day 3, and patient #3-day 7); (5) use an alternative method of vector delivery (small catheter); (6) delete adenosine administration, (7) replace skin patch with subcutaneous needle for nasal epithelium voltage measurements; (8) decrease the period of hospitalization from 2-3 weeks to 5-7 days; and (9) eliminate nasal brushings.
11/3/93	12/2/93	9209-030	Deisseroth, Albert B.	(1) Addition of PIXY321 growth factor during retrovirus transduction, and (2) perform stromal monolayers 2 weeks before retrovirus transduction.
11/18/93	12/2/93	9209-030	Deisseroth, Albert B.	Expand patient eligibility criteria to include indolent non-Hodgkin's lymphoma (NHL) patients. The revised protocol title reads: Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients
11/29/93	4/16/93	9212-034	Crystal, Ronald G.	(1) Expand the CFTR genotype eligibility criteria from the delta F508 genotype to "any CFTR genotype", and (2) delete the 3-day waiting period between the baseline and vehicle control periods and between the vehicle control and experimental treatment periods.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	MINOR MODIFICATION
11/29/93	8/14/92	9206-019	Oldfield, Edward H.	The number of patients accrued onto the study will remain the same; however, the numerical constraints as to each diagnostic group (primary versus metastases) will be eliminated.
1/6/94	8/14/92	9206-023	Dunbar, Cynthia	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
1/6/94	8/14/92	9206-024	Dunbar, Cynthia	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
1/6/94	8/14/92	9206-025	Dunbar, Cynthia	The transduction conditions may be modified as follows: (1) cells obtained from 3 patients will be transduced for 6 hours without the addition of exogenous growth factors, and (2) cells obtained from 3 patients will be transduced for 72 hours in the presence of previously isolated autologous bone marrow stromal cells without the addition of exogenous growth factors.
2/4/94	4/16/93	9212-034	Crystal, Ronald G.	(1) The protocol may be conducted at additional sites (the University Hospital, Rockefeller University, New York, New York, and the New York Hospital-Cornell Medical Center, New York, New York); and (2) nasal administration of the AdCFTR vector, without lung administration, may be conducted on an additional 14 patients; and (3) the dosing schedule may be modified in accordance with FDA requirements.
2/17/94	9/6/90	9007-003	Rosenberg, Steven A.	The transmembrane portion of the tumor necrosis factor gene may be replace with the γ-IFN signal peptide.
2/17/94	10/7/93	9303-042	Boucher, Richard C. Knowles, Michael R.	(1) Cystic fibrosis patients and healthcare workers will not be required to demonstrate neutralizing antibodies to adenovirus type 5 (Western blot and ELISA confirmation will be maintained), and (2) an additional 3 patients may be accrued onto the study at a lower starting dose of 10^7 pfu/ml.
2/17/94	2/10/94	9312-067	Welsh, Michael J.	A duplicate protocol may be initiated at the University of Washington, Seattle, Washington, in addition to the University of Iowa College of Medicine, Iowa City, Iowa. The PI will be responsible for quality control and semi-annual data reporting for research conducted at both locations.
2/25/94	12/2/93	9306-044	Deisseroth, Albert	(1) IL-3 and IL-6 may be added during the ex vivo transduction procedure, and (2) the eligibility criteria may be modified.
2/25/94	12/2/93	9306-044	Deisseroth, Albert	(1) Patient eligibility will be limited to patients with stage III/IV ovarian cancer; (2) patients who have received carboplatin (doses <300mg/m^2 <2 weeks prior), may be excluded; (3) patient accrual will be limited to 10 fully evaluable patients (max. of 20 patients entered); (4) exclusion of patients with >grade 1 neuropathies; and minor revisions to the protocol and Informed Consent document.
2/25/94	2/5/93	9209-027	Schuening, Friedrich	(1) Revised title is: A Phase I/II Study of PIXY321, a rhGM-CSF/rhIL-3 Fusion Protein or rhG-CSF, for the Mobilization of Peripheral Blood Stem Cells for Autologous Stem Cell Transplantation; (2) rhIL-3/GM-CSF (PIXY321) or rhG-CSF will be used to mobilize peripheral blood stem cells instead of rhIL-3; (3) peripheral blood stem cells alone may be used for transplantation instead of peripheral blood stem cells and additional bone marrow cells; (4) patient eligibility is modified to include non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, breast cancer, and other solid tumors; and (5) the cytoreduction chemotherapy regimen may be modified.
2/25/94	2/5/93	9209-028	Schuening, Friedrich	This study will be merged with protocol # 9209-027; therefore, this protocol is considered closed.

DATE OF MINOR MODIFI-CATION	ORIGINAL PROTOCOL APPROVAL (NIH)	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	MINOR MODIFICATION
3/23/94	12/2/93	9309-053	Cassileth, Peter Podack, Eckhard Savaraj, Niramol Sridhar, Kasi	The transfection protocol may be modified to include calcium/phosphate precipitation and DEAE dextran as optional procedures in addition to Lipofection
3/23/94	8/14/92	9206-023	Dunbar, Cynthia	(1) The University of Massachusetts Medical Center, Worcester, Massachusetts, may be included as an additional site for this study. The total number of patients approved by the RAC (12) will be divided between the NIH and the University of Massachusetts Medical Center. (2) Exogenous growth factors may be eliminated from the transduction procedure.
3/23/94	8/14/92	9206-025	Dunbar, Cynthia	(1) The University of Massachusetts Medical Center, Worcester, Massachusetts, may be included as an additional site for this study. The total number of patients approved by the RAC (12) will be divided between the NIH and the University of Massachusetts Medical Center. (2) Exogenous growth factors may be eliminated from the transduction procedure.
4/11/94	4/16/93	9212-034	Crystal, Ronald G.	(1) The criteria for nasal administration may be modified from 1.2 liters/second to 0.8 liters/second, (2) the patient eligibility criteria may be modified to allow concurrent DNAse therapy, and (3) the following inclusion criterion may be deleted, "no evidence of replication of AdCFTR DNA upon in vitro infection of the patient's respiratory epithelium with AdCFTR".
4/13/94	4/16/93	9303-041	Wilmott, Robert Whitsett, Jeffrey Trapnell, Bruce	(1) There will be a total of 15 patients, 5 cohorts with 3 individuals per cohort. Each group will receive nasal and lung administration of the following doses: $1x10^6$ CFU, $3.3x10^6$ CFU, $1x10^7$ CFU, $3.3x10^7$ CFU, and $1x10^8$ CFU. (2) The adenovirus vector volume will be reduced to 10 ml. (3) Instillation of a control vehicle in the nostril and lung of non-treated patients. (4) Addition of 5 ml saline nasal lavage. (5) Extend follow-up evaluations from 20 to 52 weeks. (6) Reduce the number of chest x-rays. (7) Delete vector coating of the trachea. (8) A patient advocate will be assigned to every patient. (8) Inclusion criteria will be modified for female patients using oral contraceptives to include barrier contraception. (10) Patients must have 2 CFTR mutations. (11) Modify exclusion criteria to include patients with active infection by viruses known to complement E1/E3-deficient adenovirus vectors, presence of allergic bronchopulmonary aspergillosis, cor pulmonale, clinical asthma, insulin-dependent glucose intolerance, current major atelectasis, chronic colonization of lower airways by Pseudomonas cepacia, or PCR detectable E1A sequences in nasal epithelial cells.
6/1/94	9/3/93	9209-020	Walker, Robert	(1) In accordance with FDA requirements, patients will receive unfractionated gene-marked lymphocytes; therefore, lymphocytes will not be separated into CD4(+) and CD8(+) subpopulations and marked separately. (2) The inclusion criteria will be modified such that patients who demonstrate a baseline CD4(+) count below 500 cells/mm^3 or who experience viral activation in association with cell infusion, must receive antiretroviral therapy. (3) T cells will be marked with either LNL6/Neo^R or G1Na/Neo^R, not both vectors.

ORDA/June 8, 1994

ACRONYMS

α	alpha
ABMT	autologous bone marrow transplantation
β	beta
BL	biosafety level
CEA	carcinoembryonic antigen
CF	cystic fibrosis
CML	chronic myelogenous leukemia
CMV	cytomegalovirus
CT	computed tomography
CTL	cytotoxic T lymphocyte
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FACC	Fanconi anemia complementation group C
FDA	Food and Drug Administration
GAG	glycosaminoglycan
G-CSF	granulocyte colony stimulating factor
GCV	ganciclovir
GVH	graft-versus-host
HLA	human leukocyte antigen
HSV-TK	herpes simplex virus thymidine kinase
IBC	Institutional Biosafety Committee
IL	interleukin
IRAP	interleukin-1 receptor antagonist protein
IRB	Institutional Review Board
IRES	internal ribosome entry sites
kg	kilogram
MDR-1	multidrug resistance type-1
μg	microgram
mg	milligram
MHC	major histocompatibility complex
ml	milliliter
mRNA	messenger ribonucleic acid
NCI	National Cancer Institute
neo^R	neomycin resistance
NIH	National Institutes of Health
ng	nanogram
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NSCLC	non-small cell lung cancer
OPRR	Office for Protection from Research Risks
ORDA	Office of Recombinant DNA Activities
PCR	polymerase chain reaction
PFU	plaque forming units
PI	principal investigator

Points to Consider	*Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects*
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
TIL	tumor infiltrating lymphocytes
UCLA	University of California, Los Angeles
USDA	U.S. Department of Agriculture
VCAM	vascular cell adhesion molecules

RECOMBINANT DNA ADVISORY COMMITTEE (RAC)
DATA MANAGEMENT REPORT - JUNE 1994*

Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
8810-001[a]	Rosenberg/ NIH	10-3-88	3-2-89	M	Cancer	5-22-89	LNL6/Neo^R	PA317	GTI	Unlimited	10	M-Yes	NA	No	No	9	Open[d]
9007-002[a,b]	Blaese/ NIH	7-31-90 2-10-92[b]	9-6-90 4-22-92[b]	T	SCID (ADA-defic.)	9-14-90 T cells 5-13-93 PBSC	LASN-ADA/ Neo^R G1NaSvAd/ Neo^R	PA317	GTI	10	6 (2 T cells, 1 PBSC, and 3 CB/PC)	T-YesG/YesP (T cells) T-YesG/NoP (PBSC) T-YesG/YesP (CB/PC)	ND	No	No	0	Open
9007-003[a]	Rosenberg/ NIH	7-31-90	9-6-90	T	Cancer	1-29-91	TNFNeo^R/ pLT12SN	PA317	GTI/ Chiron	50	12	T-YesG/YesP	Yes	No	1-transient elevation of liver function	9	Open
9102-004[a]	Brenner, et. al./ St. Jude	2-4-91	7-12-91	M	BMM/ AML	9-9-91	LNL6/Neo^R G1Na/Neo^{R,a}	PA317	GTI	12	12	M-Yes	NA	No	No	4	Closed (11-15-93)
9105-005[a]	Brenner, et. al./ St. Jude	5-31-91	7-12-91	M	BMM/ NB	1-27-92	LNL6/Neo^R G1Na/Neo^{R,a}	PA317	GTI	10	6	M-Yes	NA	No	No	1	Closed (6-1-92)
9105-006[a]	Brenner, et. al./ St. Jude	5-31-91	7-12-91	M	BMM/ NB	1-16-92	LNL6/Neo^R G1Na/Neo^{R,a}	PA317	GTI	10	3	M-Yes	NA	No	No	2	Closed (4-9-93)
9105-007[a]	Deisseroth/ MD Anderson	5-31-91	7-12-91	M	BMM/ CML	7-31-92	LNL6/Neo^R	PA317	GTI	10	5 transplanted /3 evaluable	M-Yes	NA	1/9 insufficient marking	ND-2/5 failed to engraft	3/5	Closed (6-1-93)
9105-008	Ledley, et.al./ Baylor	5-30-91	7-12-91	M	Acute Hepatic Failure	NA	LNL6/Neo^R	PA317	GTI	6	0	NA	NA	NA	NA	NA	Closed (Never Initiated)
9105-009[a]	Lotze/ U. Pittsburgh	5-30-91	1-17-92	M	Cancer/ Melanoma	3-3-92	LNL6/Neo^R	PA317	GTI	20	4	M-Yes	NA	No	No	1	Open
9110-010[a]	Rosenberg/ NIH	10-7-91	10-15-91	T	Cancer	10-8-91	TNF-Neo^R/ pLT12SN	PA317	GTI/ Chiron	5	3	V-NA	No	No	No	3	Open[d]
9110-011	Rosenberg/ NIH	10-7-91	10-15-91	T	Cancer	3-12-92	G1NaCVi2	PA317	GTI	5	2	V-NA	No	No	No	2	Open[d]
9110-012[a]	Wilson/ U. PA	10-8-91	11-14-91	T	FH	6-5-92	CMV-BA-(h)LDLR	Ψ-crip	U. PA	5	5	T-YesG/YesP	Yes (Published)	No	No	0	Closed (3-11-94)

Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9202-013[a]	Nabel/ U. MI	2-10-92	4-17-92	T	Cancer	6-3-92	HLA-B7/β-2 microglobulin (liposomes)	NA	Vical	12	5	T-YesG/YesP	Yes (Published)	No	No	5	Closed (11-19-92)
9202-014	Cornetta/ IN U.	2-11-92	4-17-92	M	BMM/ AML ALL	4-23-92	G1Na/Neo^R	PA317	GTI	20 (10 AML and 10 ALL)	7/5 evaluable (4 AML and 1 ALL)	M-Yes	NA	No Sustained Marking	No	2	Open
9202-015	Economou/ UCLA	2-11-92	4-17-92	M	Cancer/ Melanoma and Renal Cell	2-19-93	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	40 (20 Melanoma and 20 Renal Cell)	9	M-Yes	NA	No	No	3	Open
9202-016[a]	Freeman/ Tulane U.	2-10-92	2-5-93	T	Cancer/ Ovarian	8-93	HSV-TK	PA317	U. Rochester	20	2	T-NE	No	No	Yes-fever, nausea, vomiting, abdominal pain	1	Open
9202-017[b]	Greenberg, et. al./ FHCRC	2-11-92 9-9-93[b]	4-17-92 10-7-93[b]	M	HIV(+)	2-26-93	HyTK	PA317	TGC	30 (15 NHL/ HIV+ and 15 HIV+)	3 (1 NHL BMT recipient and 2 non-BMT)	M-Yes (Transient Only)	NA	No	No	1	Open
9206-018	Brenner, et. al./ St. Jude	6-1-92	8-14-92	T	Cancer/ NB	12-9-92	G1NaCvi2	PA317	GTI	12	3	V-NA (Transient Only)	No	Yes-inefficent trans-duction	No	3	Open
9206-019	Oldfield/ NIH	6-1-92	8-14-92	T	Cancer/ Brain	12-7-92	G1TkSvNa	PA317	GTI	20	15	T-YesG/YesP	Yes	Low trans-duction efficiency	Yes-hemorrhage/ toxicity in 2 patients	7	Open
9206-020[a]	Deisseroth/ MD Anderson	6-2-92	8-14-92	M	BMM and PBM/ CML	12-17-93	G1Na/Neo^R	PA317	GTI	10	4	M-P	NA	Insufficent Marking	No	1	Open
9206-021	Gansbacher, et. al./ MSKCC	6-2-92	8-14-92	T	Cancer/ Melanoma	2-17-93	NAPAD/IL2	GP+env AM12	MSKCC	12	10	V-NA	Yes-stable disease in 1 patient	No	Yes-Grade 1 induration and erythema	6	Open
9206-022[a]	Gansbacher, et. al./ MSKCC	6-2-92	8-14-92	T	Cancer/ Renal Cell	3-17-93	NAPAD/IL2	GP+env AM12	MSKCC	12	8	V-NA	Yes-stable disease in 2 patients	No	Yes-Grade 1 induration and erythema	6	Open
9206-023[a]	Dunbar/ NIH and U. MA	6-2-92	8-14-92	M	BMM and PBM/ Multiple Myeloma	10-28-92	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	8	M-Yes (low efficiency)	NA	No	No	0	Open

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Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9206-024[a]	Dunbar/ NIH	6-2-92	8-14-92	M	BMM and PBM/ Breast Cancer	11-20-92	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	5	M-Yes (low efficiency)	NA	No	No	1	Open
9206-025[a]	Dunbar/ NIH and U. MA	6-2-92	8-14-92	M	BMM and PBM/ CML	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	NA	NA	NA	NA	NA	P/FDA
9206-026	Walker/ NIH	9-14-92	9-3-93	M	HIV+/ Identical Twin	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	NA	NA	NA	NA	NA	P/FDA
9209-027[a]	Schuening/ FHCRC	9-14-92	2-5-93	M	BMM and PBM/ solid tumors	4-10-94	LN/neo^R	PA317	TGC	20	2	M-P	NA	P	P	0	Open
9209-028	Schuening/ FHCRC	9-14-92	2-5-93	M	BMM and PBM/ Lymphoid Malig.	NA	LN/neo^R	PA317	TGC	20	0	NA	NA	NA	NA	NA	Closed (Merged with 9209-027)
9209-029	Schuening/ FHCRC	9-14-92	2-5-93	M	BMM and PBM/ Identical Twins	NA	LN/neo^R	PA317	TGC	20	0	NA	NA	NA	NA	NA	Closed (Never Initiated)
9209-030	Deisseroth/ MD Anderson	9-14-92	12-2-93	M	BMM and PBM/ CLL, follicular NHL	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	10	0	M-P	NA	NA	NA	NA	P/FDA
9403-031 9209-031	Roth/ MD Anderson	3-4-94 ~~9-15-92~~	P NA	T	Cancer/ NSCLC	P	LNAS-K-ras and LNp53B	GP+env AM12	MA	14	0	NA	NA	NA	NA	NA	P/FDA and NIH
9209-032	Brenner/ St. Jude	9-15-92	2-5-93	M	BMM/ NB	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	NA	NA	NA	NA	NA	Open[d]
9209-033	Lotze, et. al./ U. Pittsburgh	9-15-92	2-5-93	T	Cancer	9-9-93	G1ILASvNa	PA317	GTI	20	9	V-NA	NA	No	Yes-fever, chills, diarrhea, skin irritation	2	Open
9212-034[a]	Crystal/ Cornell, Rockefeller U., and NIH	12-3-92	4-16-93	T	CF	4-17-93	AdCFTR (type 5)	293	Cornell NIH	24 (10 Lung, 14 Nasal)	5	T-YesG/YesP	Yes	No	Yes-transient mild inflam-mation	0	Open
9212-035[a]	Wilson/ U. PA	12-3-92	8-26-93	T	CF	9-3-93	AD.CB-CFTR (type 5)	293	U. PA	20	7	T-P	No	No	No	0	Open

Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9212-036[a]	Welsh, et. al./ HHMI	12-4-92	4-16-93	T	CF	7-26-93	Ad2/CFTR-1 (type 2)	293	Genzyme	6	4	T-YesG/YesP	Yes (Published)	No	No	0	Open
9303-037	Culver, et. al./ IA Methodist and U. IA	3-1-93	4-16-93	T	Cancer/ Brain	P	G1TKSvNa	PA317	GTI	15	0	NA	NA	NA	NA	NA	P/FDA
9303-038	Heslop/ St. Jude	3-2-93	4-16-93	M	EBV-specific CTL/ Cancer	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	35	7	M-Yes	NA	No	Yes-mild exacer-bation of GVHD	1	Open
9303-039	Brenner, et. al./ St. Jude	3-2-93	4-16-93	M	BMM/ AML	10-12-93	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	35	3	M-Yes	NA	No	No	No	Open
9303-040	Simons/ Johns Hopkins	3-1-93	12-2-93	T	Cancer/ Renal Cell	1-24-94	MFG-hu-GM-CSF	Ψ-crip	Somatix Therapy	26	8	V-NA	No	No	No	0	Open
9303-041[a]	Wilmott, et. al./ Children's Hosp., OH	3-2-93	4-16-93	T	CF	P	Av1CF2 (type 5)	293	GTI	15	NA	NA	NA	NA	NA	0	P/FDA
9303-042[a]	Boucher, et. al./ U. NC	3-2-93	10-7-93	T	CF	1-11-94	Ad.CB-CFTR (type 5)	293	U. PA	12	4	T-YesG/NoP	No	No	No	0	Open
9306-043	Seigler/ Duke U.	6-7-93	9-3-93	T	Cancer/ Melanoma	8-10-93	pHUγ-IFN	VCHUγ	Viagene	20	5	V-NA	No	No	No	2	Open
9306-044[a]	Deisseroth, et. al./ MD Anderson	6-7-93	12-2-93	T	Cancer/ Ovarian	P	MDR-1/LN	PA317	ATCC	10-fully evaluable (max. 20 entered)	0	NA	NA	NA	NA	NA	P/FDA
9306-045	Nabel/ U. MI	6-7-93	9-3-93	T	Cancer	2-2-94	pHLA-B7/ β-2 μglobulin (liposomes)	NA	Vical	24 (12 intratumor and 12 catheter)	1	T-P	No	No	No	0	Open
9306-046	Barranger/ U. Pittsburgh	6-7-93	9-3-93	T	Gaucher Disease	P	R-GC	Ψ-crip	U. Pittsburgh	5	0	NA	NA	NA	NA	NA	Open
9306-047	Karlsson, et. al./ NIH and CHLA	6-7-93	9-3-93	T	Gaucher Disease	P	G1Gc	PA317	GTI	10 (5-ABM 5-PBL)	0	NA	NA	NA	NA	NA	P/FDA
9306-048	Galpin, et. al./ Shared Med. Res. Foundation	6-7-93	9-3-93	T	HIV(+)	8-10-93	[HIV-IT(V)]	D17	Viagene	15 (3 groups of 5)	16	T-P	No	No	No	0	Open

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Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9306-049	Nabel/ U. MI	6-7-93	9-3-93	T	HIV(+)	P	(Rev M10) (ΔRev M10) and (RSV-tar/ Rev M10) (RSV-tar/ Rev M10)	Ψ-crip	Vical	12 (4 groups of 3)	0	NA	NA	NA	NA	NA	P/FDA
9306-EXP[c]	Sobol, et. al./ SDRCC	NA	12-28-93 5-11-93[c]	T	Cancer/ glioblastoma	1-4-93	12-28-93 (NAPAD-IL2) 5-11-93 (G1NaCvi2)	GP+env AM12 PA317	MSKCC GTI	1	1	No	?	No	No	1	Closed (1-20-93 Patient Death)
9306-050	Raffel, et. al./ CHLA and IA Methodist	6-8-93	9-3-93	T	Cancer/ Astrocytoma	P	G1TkSvNa	PA317	GTI	15 (if + response- additional 15 may be accrued)	0	NA	NA	NA	NA	NA	P/FDA
9306-051	Hesdorffer, et. al./ Columbia U.	6-8-93	9-3-93	T	Cancer/ Breast, Ovarian, and Brain	P	pHAMDR1/A	GP+env AM12	MA GTI	20	0	NA	NA	NA	NA	NA	Open
9306-052	Ilan/ Case Western Reserve	6-8-93	12-2-93	T	Cancer/ Brain	P	pAnti-IGF-I (EBV derived)	NA	Case Western Reserve	12	0	NA	NA	NA	NA	NA	Open
9309-053	Cassileth, et. al./ U. Miami	9-9-93	12-2-93	T	Cancer/ Small cell lung cancer	P	pBMG-Neo-hIL2/BPV derived (liposomes)	NA	U. Miami	12	0	NA	NA	NA	NA	NA	Open
9309-054	O'Shaugnessy /NIH	9-9-93	10-7-93	T	Cancer/ Breast	P	G1MD	PA317	GTI	18	0	NA	NA	NA	NA	NA	P/FDA
9309-055	Kun, et. al./ St. Jude	9-9-93	10-7-93	T	Cancer/ Brain	P	G1TkSvNa	PA317	GTI	6	0	NA	NA	NA	NA	NA	P/FDA
9309-056	Das Gupta, et. al./ U. IL	9-10-93	4-19-94	T	Cancer/ Melanoma	P	pZipNeoSv-IL2	GP+env AM12	U. IL	12	0	NA	NA	NA	NA	NA	P/FDA
9309-057	Wong-Staal, et. al./ USD	9-10-93	P	T	HIV(+)	P	pMJT (hairpin ribozyme)	PA317	ND	6	0	NA	NA	NA	NA	NA	P/FDA and NIH
9309-058	Economou, et. al./ UCLA	9-10-93	12-2-93	T	Cancer/ Melanoma	P	G1NaCvi2	PA317	GTI	30 (3 groups of 10)	0	NA	NA	NA	NA	NA	P/FDA
9312-059	Oldfield, et. al./ NIH	12-2-93	1-20-94	T	Cancer/ Leptomeningeal Carcinomatosis	P	G1TkSvNa	PA317	GTI	20	0	NA	NA	NA	NA	NA	Open

Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9312-060	Sobol, et. al./ SDRCC	12-2-93	P	T	Cancer/ Colon	P	LNCX/IL2	PA317	?	12	0	NA	NA	NA	NA	NA	P/FDA and NIH
9312-061	Schuening/ FHCRC	12-2-93	P	T	Gaucher Disease	P	LgGC	PG13	FHCRC	10	0	NA	NA	NA	NA	NA	P/FDA and NIH
9312-062	Haubrich/ U. CA at San Diego Treatment Center	12-2-93	4-19-94	T	HIV(+)	P	HIV-IT(V)	D17	Viagene	25	0	NA	NA	NA	NA	NA	Open
9312-063	Sznol/ NIH	12-3-93	4-19-94	T	Cancer/ Melanoma	P	BCMGNeo-B7	?	NIH	48 (24 HLA-A1+ and 24 HLA-A2+)	0	NA	NA	NA	NA	NA	Open
9312-064	Rubin/ Mayo Clinic	12-3-93	4-19-94	T	Cancer/ Colon	P	pHLA-B7/ β-2 microglobulin (liposomes)	NA	Vical	15	0	NA	NA	NA	NA	NA	Open
9312-065	Chang/ U. MI	12-3-93	P	T	Cancer/ Melanoma	P	GBAH4	Ψ-crip	MA	15	0	NA	NA	NA	NA	NA	P/FDA and NIH
9312-066	Sorscher, et. al/ U. AL	12-3-93	P	T	CF	P	pKCTR (liposomes)	NA	Vical	9	0	NA	NA	NA	NA	NA	P/FDA and NIH
9312-067	Welsh/ HHMI and U. WA	12-3-93	2-10-94	T	CF	P	Ad2-ORF6/PGK-CFTR and AD2/CFTR-1 (type 2)	293	Genzyme	40 (20 HHMI and 20 U. WA)	0	NA	NA	NA	NA	NA	Open
9403-068	Rosenblatt, et. al./ UCLA and CHLA	3-3-94	P	T	Cancer/ Neuroblastoma	P	pHUγ-IFN	VCHU	Viagene	18	0	NA	NA	NA	NA	NA	P/FDA and NIH
9403-069	Walker/ NIH	3-3-94	P	T	HIV(+)	P	ζrkat4SVGD3e-	PA317	Cell Genesys	x-single dose 40-multiple dose	0	NA	NA	NA	NA	NA	P/FDA and NIH
9403-070	Brigham/ Vanderbilt U.	3-3-93	P	T	AAT-Deficiency	P	pCMV4-AAT (liposomes)	NA	Vander-bilt U.	11 (6 Nasal and 5 Lung)	0	NA	NA	NA	NA	NA	P/FDA and NIH

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Protocol #	PI(s)/ Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patients Entered onto Study	In Vivo Evidence of Gene Transfer	Biological Efficacy	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9403-071	Vogelzang/ U. Chicago	3-4-94	4-10-94	T	Cancer/ Renal Cell	P	pHLA-B7/ β-2 microglobulin (liposomes)	NA	Vical	15 evaluable	0	NA	NA	NA	NA	NA	Open
9403-072	Hersh, et. al./ AZ Cancer Center	3-4-94	4-19-94	T	Cancer/ Melanoma	P	pHLA-B7/ β-2 microglobulin (liposomes)	NA	Vical	15 evaluable	0	NA	NA	NA	NA	NA	Open
Total # Patients											219						

* Current data as of May 3, 1994.

TABLE LEGEND:

a	= Minor Modification
b	= Major Amendment
c	= Expedited Review
d	= No Patients Entered On the Study > 1 Year
M	= Gene Marking Protocol
M-No	= Gene Marking Protocol/Marked Cells Not Detected In Vivo (In Vitro Marking Successful)
M-P	= Gene Marking Protocol/Data Pending
M-Yes	= Gene Marking Protocol/Marked Cells Detected In Vivo
NA	= Not Applicable
ND	= Not Determined
NE	= Not Evaluable
No Marking	= Gene Marking Protocol/No Evidence of Successfull Marking
P	= Pending
T	= Gene Therapy
T-NoG	= Gene Therapy Protocol/No Evidence of Gene Transfer In Vivo
T-P	= Gene Therapy Protocol/Data Pending
T-YesG/YesP	= Gene Therapy Protocol/Evidence of Gene Transfer In Vivo/Evidence of Protein Expression
T-YesG/NoP	= Gene Therapy Protocol/Evidence of Gene Transfer In Vivo/No Evidence of Protein Expression
V-NA	= Vaccine Protocol/Evidence of Gene Transfer Not Applicable

	T	M	Total (T + M)
RAC Approved	50	22	72
NIH Director Approved	41	22	63
Categories of RAC-Approved Protocols			
Cancer	32	4	36
Cystic Fibrosis	7	0	7
SCID/ADA	1	0	1
Acute Hepatic Failure	0	1	1
Familial Hypercholesterolemia	1	0	1
Gaucher Disease	3	0	3
Bone Marrow Marking/Cancer	0	15	15
HIV(+)	5	2	7
Alpha-1-Antitrypsin Deficiency/Acute Lung Injury	1	0	1

ACRONYMS:

AAT	= Alpha-1 Antitrypsin
ADA	= Adenosine Deaminase
ABM	= Autologous Bone Marrow
ALL	= Acute Lymphocytic Leukemia
AML	= Acute Myelogenous Leukemia
ATCC	= American Type Culture Collection
BMM	= Bone Marrow Marking
BPV	= Bovine Papilloma Virus
CB	= Cord Blood
CF	= Cystic Fibrosis
CFU	= Colony Forming Units
CHLA	= Children's Hospital Los Angeles
CLL	= Chronic Lymphocytic Leukemia
CML	= Chronic Myelogenous Leukemia
CTL	= Cytotoxic T Lymphocytes
EBV	= Epstein-Barr Virus
FDA	= Food and Drug Administration
FH	= Familial Hypercholesterolemia
FHCRC	= Fred Hutchinson Cancer Research Center
G-CSF	= Granulocyte - Colony Stimulating Factor
GC	= Glucocerebrosidase
GTI	= Genetic Therapy, Inc.
GVHD	= Graft Versus Host Disease
HHMI	= Howard Hughes Medical Institute
HIV	= Human Immunodeficiency Virus
MA	= Microbiological Associates
MSKCC	= Memorial Sloan-Kettering Cancer Center
NB	= Neuroblastoma
NHL	= Non-Hodgkin's Lymphoma
NIH	= National Institutes of Health
NSCLC	= Non-Small Cell Lung Cancer
PBL	= Peripheral Blood Lymphocytes
PBM	= Peripheral Blood Marking
PBSC	= Peripheral Blood Stem Cells
PC	= Placenta Cells
SCID	= Severe Combined Immunodeficiency
SDRCC	= San Diego Regional Cancer Center
TGC	= Targeted Genetics Corporation
UCLA	= University of California, Los Angeles
USC	= University of Southern California

Submitted June 94
Date 17, 1994

HUMAN GENE TRANSFER PROTOCOL

9406-073 Curiel, David, University of Alabama, Birmingham, Alabama; *Phase I Trial of a Polynucleotide Augmented Anti-tumor Immunization to Human Carcinoembryonic Antigen in Patients with Metastatic Colorectal Cancer.*

Date of RAC Approval: 6/9/1994

Scientific Abstract

Metastatic colorectal adenocarcinoma is a rapidly fatal disease in the vast majority of affected patients despite combined modality therapy with radiation and chemotherapy. Strategies have been developed to attempt to eradicate malignant cells by stimulating a host immune response to the aberrant colon cancer cells. In this regard, a recombinant vaccinia viral vector encoding human carcinoembryonic antigen (CEA), a relevant tumor-associated antigen, is undergoing clinical trials with the goal of eliciting CEA-specific immune responses. It is the purpose of this protocol to evaluate the feasibility of eliciting CEA-specific immunity utilizing the strategy of polynucleotide vaccination. Extensive work has shown that naked plasmid DNA injected into muscles is taken up by myocytes with expression of encoded genes. Further, the expression of these genes can elicit humoral and cellular immune responses to encoded antigens. We have shown in murine models that a CEA polynucleotide vaccine can successfully elicit antibody and T cell immune responses to human CEA. Further, studies have determined threshold and optimal dosing regimens. The CEA polynucleotide vaccine has also been demonstrated to protect animals against challenge with a syngeneic murine colon carcinoma line expressing human CEA. In this present context, we will deliver a plasmid encoding the cDNA for human CEA. This plasmid will be injected intramuscularly to determine whether anti-CEA immunity can be elicited in humans by this route. It will further be determined whether this route of gene transfer to humans is safe and associated with any toxicity.

Non-Technical Abstract

Metastatic cancer of the colon or rectum is a rapidly fatal disease in the vast majority of affected patients despite combined modality therapy with radiation and chemotherapy. There is a large body of evidence in animal systems indicating that the immune system, including antibodies and immune cells termed lymphocytes, can be stimulated to recognize and destroy cancer cells. Experimental studies have been carried out in humans to attempt to induce these immune responses by means of a "vaccine." In this strategy, the patient is challenged with a vaccine which presents to the immune system the factor or factors (tumor antigen) which may allow the cancer cells to be recognized as "foreign" and rejected by the immune system. In the present study, antitumor immune responses will be elicited by a "polynucleotide vaccine." The polynucleotide vaccine consists of a highly purified and well defined DNA molecule which promotes expression of carcinoembryonic antigen (CEA), a protein found selectively on colon cancer cells and which flags these cells as abnormal to the immune system. The DNA is injected into the muscle of the patient where expression of the CEA gene induces an immune response to CEA. The immune response generated to the DNA expressed CEA should then allow recognition by the immune system of tumor cells containing CEA. This study will evaluate the safety of this method of gene delivery. Additionally, it will be determined whether this method of vaccination can successfully elicit an immune response to colon cancer cells in humans.

COMPREHENSIVE CANCER CENTER
UNIVERSITY OF ALABAMA AT BIRMINGHAM
BIRMINGHAM, ALABAMA

Protocol

PROTOCOL:UAB 9405 - **Phase Ia Trial of a Polynucleotide Vaccine to Human Carcinoembryonic Antigen in Patients with Metastatic Colorectal Cancer.**

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Approval Dates _____ _____
CSRC IRB

David T. Curiel, M.D. NCI (Pending)
Principal Investigator Study Sponsor

Albert F. LoBuglio, M.D.
Robert M. Conry, M.D. Polynucleotide Vaccine for CEA
Co-Investigators Study Agent(s)

_____
Data Coordinator

ADDITIONAL SITES: BVAH - YES / NO AFFILIATES - YES / NO

1.0 SPECIFIC AIMS

In patients with metastatic colorectal cancer, to:

1.1 Characterize the immune response to carcinoembryonic antigen (CEA) after single immunization with a polynucleotide vaccine composed of the plasmid DNA of CEA.

1.2 Characterize the immune response to CEA after three repetitive doses of the CEA polynucleotide vaccine.

1.3 Characterize the toxic or adverse effects associated with single and repetitive administration of the CEA polynucleotide vaccine given over a dose range of 0.1 - 1.0 mg (single dose) and 0.9 - 3.0 mg (total dose) in a multi-dose regimen.

2.0 BACKGROUND AND RATIONALE

2.1 Humans are able to generate an immune response to potential tumor relevant antigens following immunization with tumor vaccines.

There is a long history of clinical trials using various tumor preparations or extracts to immunize cancer patients. In general, these have represented rather crude attempts at active specific immunotherapy with poorly defined immunogens and less than optimal monitoring assays. These problems reflected the state-of-the-art in human tumor immunology and not a shortcoming of the clinical investigators. Despite these problems, some patients had clinical evidence of anti-tumor effects. More recently, several vaccine trials have provided more definitive evidence of vaccine-induced anti-tumor responses. Several examples involve malignant melanoma studies (1,2,3). The studies by Mastrangelo's group (1,4) have utilized autologous, enzyme disassociated, irradiated tumor cells plus BCG to several sites three days following bolus cytoxan. Immunizations were repeated Q4 weeks with observed regression of metastatic tumor sites and evidence of delayed hypersensitivity skin test reactivity to autologous tumor cells. Mitchell's group (2,5) has immunized metastatic melanoma patients with allogeneic melanoma cell line lysates in DETOX (adjuvant) given weekly times 4 and week 6. Patients developed evidence for delayed hypersensitivity to the immunizing lysate and cytolytic T cell precursors to one of the two original allogeneic melanoma cell lines they used "cold inhibition" studies to address issues of specificity. Objective tumor responses were reported in 4 of 25 (2) and 5 of 17 patients (5). Bystryn's group (3,6,7) has immunized low tumor burden melanoma patients with a vaccine prepared from material shed into the supernate of four different allogeneic melanoma cell lines plus alum (adjuvant) with evidence for cellular (6) and humoral response (7) to components of the vaccine and some degree of efficacy (3).

More pertinent to this proposal, similar observations have been made in colon cancer patients (8,9,10). Hollingshead (8) reported that low tumor burden colon cancer patients immunized with a partially purified "tumor-associated antigen" preparation derived from allogeneic colon cancer sonicated membrane extracts mixed with complete Freund adjuvant and administered at multiple sites monthly times 3 could induce an immune response as determined by delayed hypersensitivity skin tests and migration inhibition assays to the "tumor-associated antigens." Hoover and Hanna (9,11,12) utilized a similar group of low tumor burden (Dukes' B and C) colon cancer patients who received autologous, enzyme disassociated, live, irradiated tumor cells (10^7) mixed with BCG weekly times 2 and tumor cells alone week 3. This randomized trial provided evidence for delayed hypersensitivity skin tests (12) to autologous tumor cells (versus normal mucosal cells) and a reduced relapse rate (9,11). A very interesting and provocative recent report by the Memorial Sloan-Kettering group (10) utilized vaccine composed of a partially desialylated bovine submaxillary gland mucin alone (n=6) with DETOX (n=8) or with BCG (n=6) in colorectal cancer patients with low tumor burden (Dukes' B, C and D with no evidence of tumor). This preparation contains TN and sTN antigens expressed on various adenocarcinomas. The sTN is the epitope for B72.3 and CC49 monoclonal antibodies. This trial reported human antibody responses (IgM and IgG) in patients receiving the mucin plus adjuvant and not in the mucin alone group. All patients received pre-therapy with cytoxan. Only 1 patient developed a positive delayed hypersensitivity skin test.

There are numerous other trials with these and other tumor types which provide similar observations. Overall, it seems reasonable to say that some patients receiving active specific therapy with tumor vaccines have had evidence of immune responses and occasional tumor regressions or other evidence of efficacy. However, the antigens involved are ill defined, measures of immune response are primarily skin tests and some antibody response data. Methodology to evaluate T cell responses which are so prominent in animal model systems of active specific immunotherapy (13,14) is seldom included or not possible.

2.2 CEA represents a reasonable tumor relevant antigen for a tumor vaccine.

Carcinoembryonic antigen (CEA) is an oncofetal antigen present predominantly in fetal gutand adenocarcinomas of the colon, breast, lung, etc. It has also been identified in small amounts in normal adult colonic mucosa. CEA is a 180Kd glycoprotein expressed on the cell surface and is probably the most extensively characterized tumor-associated antigen in man (15,16). The CEA gene family belongs to the immunoglobulin super gene family and resides on the long arm of chromosome-19. Other members of this family which share significant homology with CEA (e.g., non-specific cross-reacting antigen or NCA) are found in other normal tissues including gallbladder and leukocytes (16,17). A large number of monoclonal antibodies to CEA and other family members have allowed extensive epitope mapping which define CEA specific epitopes (antigenic sites) as well as cross-reactive epitopes and epitopes specific for other family members (18).

Recently, Shively's group (19) has expressed the individual CEA domains in HeLa cells for epitope mapping purposes.

It is clear that CEA can function as an effective tumor vaccine target in an animal model of CEA expressing murine colon carcinoma line (20). Vaccination with rV-CEA produced both humoral and cellular immune responses with resultant *in vivo* anti-tumor effects. It is pertinent that mice have the CEA gene family of molecules which share 50 to 60% homology with human CEA (16) and yet the mice suffered no toxic effects. This serum tumor marker is widely used in clinical medicine and CEA is expressed in >90% of colorectal tumors in man (also found in other tumor types like breast and non-small cell lung cancer). The small amounts of this glycoprotein in normal tissue supports the view that humans have tolerance to it. However, its immunogenicity in man is controversial with reports suggesting that colon and breast cancer patients have antibody to CEA (21,22) while other reports deny the presence of specific antibody (23,24). The issues of tolerance, cross-reacting antigens and immunogenicity will be serious concerns for this and almost any human tumor relevant antigen.

2.3 Polynucleotide vaccines composed of plasmid DNA constructs of relevant genes when administered intramuscularly can induce gene expression in muscle with resultant host immune response and protective immunity.

In 1990, myofiber cells in the mouse were shown to express foreign genes that have been injected into muscle in the form of naked DNA without any cationic lipids, retroviruses or other special delivery systems (25). Such expression has also been demonstrated after injection of naked DNA into the skeletal muscle of fish (26), rats (27), cats, and rhesus monkeys (28). Expression in muscle has been demonstrated with DNA encoding reporter genes (25), dystrophin (29), and influenza nucleoprotein (30). The duration of gene expression in skeletal muscle using an RSV promoter driving a luciferase reporter exceeded 19 months post-injection, and the foreign plasmid DNA appeared to remain episomal (31). Injection of plasmid DNA into other murine tissues such as brain, liver, spleen, uterus, stomach, lung, or kidney did not produce expression (32). Pre-treatment of striated muscle with the local anesthetic bupivacaine significantly increases gene expression following naked plasmid DNA injection. This has been attributed to uptake and expression of the plasmid by a greater number of muscle fibers (33,34).

Naked plasmid DNA encoding influenza A nucleoprotein (NP) delivered to mice by IM injection produced influenza NP-specific antibodies and cytolytic T cell response with protection from subsequent challenge with influenza A virus (30). Other examples of immunization using naked plasmid DNA include induction of antibody response to human growth hormone following administration of DNA-coated micro-projectiles into mouse skin (35); induction of neutralizing (hemagglutination inhibiting) antibodies by injection of DNA encoding influenza A hemagglutinin in mice, ferrets and rhesus monkeys (36) with protective immunity to challenge (37); antibody response to bovine herpes virus in mice and

cattle (38); and circulating antigen followed by antibody response to hepatitis B-surface antigen in mice (39). Our own group has demonstrated immune response to human carcinoembryonic antigen using intramuscular injection of plasmid DNA in mice (40).

2.4 Immune response to CEA can occur in mice and non-human primates and confer both immunoprotection and therapy in syngeneic CEA expressing murine tumor models.

Schlom's group (41) described the cloning of the CEA gene from a human colon tumor cell library. The 2.4 Kb cDNA clone contained the complete coding sequence for CEA and was successfully inserted into a vaccinia virus genome (NYC attenuated strain). This group (42) inserted the cDNA clone for CEA into the murine colon carcinoma cell line, MC-38, and demonstrated that this tumor was now CEA positive with cell surface display of intact CEA (180 Kd). The recombinant virus vaccine (rV-CEA) induced high titers of IgG antibodies to CEA; delayed hypersensitivity skin test reactivity and lymphoblastic transformation to CEA; cytolytic T cell responses specific for CEA positive MC-38 tumor cells; immunoprotection against challenge with 2×10^5 MC-38/CEA tumor cells; and anti-tumor effects when vaccine therapy began 7 days following challenge with 2×10^5 MC-38/CEA cells (20). These effects were all CEA specific clearly showing that this molecule can function as an effective target for tumor immunity. There were no toxic effects in mice despite the presence of homologous CEA family members in normal rodent tissues (16). We have preliminary data to indicate that CEA polynucleotide vaccines can provide immunoprotection against the same MC-38/CEA tumor challenge (2×10^5 cells). Schlom's group also examined the immunogenicity of the rV-CEA construct in primates (43). Adult rhesus monkeys received immunizations with the rV (NYC)-CEA and developed specific anti-CEA antibody responses, delayed hypersensitivity skin tests to CEA and lymphoblastic transformation to CEA. These animals have NCA (non-specific cross-reacting antigen) on their granulocyte surface. No toxicity or granulocytopenia was noted and no antibody to NCA was detected.

Thus, the pre-clinical data demonstrate the defined molecular nature of this tumor-associated antigen with solid evidence of its immunogenicity in mice and non-human primates. No toxicity has been noted in these models in regard to cross-reactivity with other members of the CEA family residing in normal tissues.

Based upon these pre-clinical observations, a phase I trial of recombinant vaccinia-CEA in patients with advanced colorectal adenocarcinoma is ongoing at the Clinical Research Center at NIH with immunologic monitoring conducted by the UAB Immunology Laboratory directed by Dr. Robert M. Conry of this protocol. Twenty patients have received single and multiple immunizations without toxicity. A phase Ib trial of recombinant vaccinia-CEA in patients with resected Dukes' C adenocarcinoma of the colon (who have completed adjuvant 5-FU/levamisole) is ongoing at the UAB Cancer Center. This trial will examine

the effects of repeated immunization with and without pre-treatment with cytoxan on CEA specific immune responses (also to be conducted by Drs. Conry and LoBuglio).

2.5 Pre-clinical studies with a polynucleotide vaccine composed of the plasmid DNA construct for human CEA can induce immune response and protection against tumor challenge in a syngeneic murine adenocarcinoma model expressing human CEA.

We have carried out pre-clinical studies in a murine model of syngeneic colon adenocarcinoma (40) which have shown that a polynucleotide vaccine composed of the c-DNA for CEA can induce humoral immunity and cellular immunity (lymphoblastic transformation and lymphokine release to CEA) to human CEA. We have subsequently tested individual doses ranging from 10 μg to 250 μg while the intramuscular injection of 50 μg three times per week for a total of 18 injections produced no evidence of local toxicity or inflammation at the injection site or systemically. Mice immunized with any one of a number of doses and schedules had evidence of immune response approximately 4 to 6 weeks post-immunization with immunoprotection against tumor challenge with 2×10^5 tumor cells given from 3 to 8 weeks post-start of vaccination. These immune responses and protection against tumor challenge were of similar magnitude as that seen with recombinant vaccinia-CEA studies described above. The intramuscular injection of a plasmid containing the cDNA for chloramphenicol acetyltransferase produced no CEA immune responses and did not protect against tumor challenge.

2.6 Patients with CEA positive metastatic colorectal cancer patients represent reasonable candidates to test the feasibility of polynucleotide vaccination as a means to induce immune response to tumor relevant and control antigens.

Patients with metastatic colorectal cancer who have failed to respond or relapsed after 5-FU therapy regimens have <10% clinically relevant response rates to second and third line therapy options. Such patients represent reasonable populations for early phase I trials of appropriate anti-tumor therapy protocols. We have utilized such patient groups in trials of monoclonal antibodies (44), ^{131}I-labeled genetically engineered monoclonal antibodies (45) and drug conjugates of monoclonal antibodies (46, protocol ongoing).

2.7 Polynucleotide vaccines should have a favorable safety profile, particularly as compared to other gene therapy strategies for cancer.

A variety of strategies have been proposed to approach cancer utilizing the techniques of gene therapy (reviewed in 47). These include genetic strategies to correct the mutational lesions characterizing cancer cells ("mutation compensation"), genetic strategies to eradicate tumor cells by encoded toxin genes ("molecular chemotherapy") and genetic strategies to enhance immune mechanisms of tumor rejection ("genetic immunopotentiation"). One of the advantages of the latter strategy is that it capitalizes on the ability of the immune

system to achieve a high level of specificity and to amplify biologic responses. Thus, unlike the first two strategies, there is no mandate to achieve genetic modification of all cancerous cells.

The stimulation of the immune system by genetic means to achieve an anti-tumor effect has been accomplished in a variety of ways. The use of gene modified autologous tumor cell vaccines (48) or genes for tumor-associated antigen incorporated into viral vaccines (vaccinia) (49) are current examples. It is also recognized that naked plasmid DNA injected into muscle can elicit immunity to proteins encoded by the plasmid DNA. This is thought to derive from the unique ability of plasmid DNA to be taken up, expressed and to achieve stable persistence when delivered in this manner. Whereas gene expression in muscles has been accomplished utilizing recombinant retrovirus and adenovirus vectors (50,51), naked plasmid DNA offers several unique advantages from the standpoint of safety. Firstly, the delivery of heterologous genes by recombinant viral vectors is associated with the obligatory co-delivery of viral gene elements. This introduces the possibility of recombinational events with other viruses to allow derivation of replication-competent recombinant viruses. Naked plasmid DNA introduced by polynucleotide vaccination can be designed in a manner to eliminate viral sequences thus overcoming this consideration. Additionally, the recombinant viruses contain sequences which may allow integration of the transferred gene into the host chromosome. This could have consequences from the standpoint of insertional mutagenesis or activation of endogenous oncogene sequences. Naked plasmid DNA delivered by direct injection into muscle has been shown to persist as an unintegrated episome (25,52). This would thus reduce the potential consequences of direct gene delivery by minimizing the likelihood of an insertional event. Further, preclinical and clinical studies of Nabel (53,54) have shown that intratumor plasmid DNA encoding major histocompatibility antigen injections in animals and man produces little or no toxicity with elicitation of anti-tumor effects. Thus, we believe that polynucleotide vaccines represent an advantageous gene therapy vaccine strategy with a favorable toxicity profile.

3.0 SCHEMA

PRE-STUDY EVALUATION

1. Eligibility check
2. Informed consent

THERAPY

Group 1
0.05 mg into each deltoid x 1
(total dose - 0.1 mg)

Group 2
0.15 mg into each deltoid x 1
(total dose = 0.3 mg)

Group 3
0.5 mg into each deltoid x 1
(total dose = 1.0 mg)

Group 4
0.15 mg into each deltoid on Days 1, 22 & 43
(total dose = 0.9 mg)

Group 5
0.5 mg into each deltoid on Days 1, 22 & 43
(total dose = 3 mg)

FOLLOW-UP

1. Toxicity monitoring on Days 1, 2, 8, 15, 29, 43, and 57.
2. Blood for humoral immune response pre-therapy and Days 15, 29, 43, and 57.
3. Blood for cellular immune response pre-therapy and Days 29 and 57.
4. Blood for CBC, Chem-18, CEA, C-reactive protein and creatine kinase on Days 1, 8, 15, 43, and 57.
5. Re-evaluation for tumor objective response on Day 57.

4.0 PATIENT SELECTION CRITERIA

4.1 Patients must have pathologically proven colon or rectal adenocarcinoma with documented CEA positive tumor cells having been resistant to or relapsed from at least one chemotherapy regimen for metastatic disease.

4.2 A minimum of 30 days should have elapsed from the time of completion of prior chemotherapy and/or radiation therapy.

4.3 Recovery from all treatment related toxicity.

4.4 No concurrent chemotherapy, radiotherapy or drugs which affect immune function such as glucocorticoids. Immunomodulatory drugs such as Cimetidine should not be administered.

4.5 Evidence of metastatic disease by a staging work-up (physician examination, chest x-ray and abdominal CT) with objectively measurable tumor masses. Chest x-rays or abdominal CT's performed within 30 days of starting treatment on study will be accepted.

4.6 A positive skin test to at least one recall antigen (e.g., mumps, candida, trichophytin) within 30 days prior to enrollment.

4.7 Patients must have the following organ system function:

Hematopoietic	Leukocyte of at least 3,500/mm^3; platelet count of at least 120,000/mm^3
Renal	BUN <30 mg%; creatinine <2 mg%.
Hepatic	Bilirubin <1.5 mg%, alkaline phosphatase <3 times normal.
Cardiac	No evidence of congestive heart failure, unstable angina or serious cardiac arrhythmias.

4.8 No prior history of malignancy, except treated basal cell, skin squamous cell cancer or carcinoma *in situ* of the cervix.

4.9 Patients must be >18 years of age.

4.10 Patients must not be pregnant. Women of child-bearing age must have a negative pregnancy test and be on an adequate contraceptive program.

4.11 No serologic evidence of hepatitis B infection.

4.12 Patients must provide written informed consent.

Exclusion Criteria

The following circumstances, if present, would classify a patient unacceptable for including in this trial:

4.13 Underlying immunodeficiency disorder or immunodeficiency state.

4.14 Active autoimmune disorder such as rheumatoid arthritis, systemic lupus erythematosis, etc. Diagnosis of altered immune function, including eczema, atopic dermatitis or autoimmune disease (autoimmune neutropenia, thrombocytopenia or hemolytic anemia; systemic lupus erythematosus, Sjogren syndrome or scleroderma; myasthenia gravis; Goodpasture syndrome; Addison's disease, Hashimoto's thyroiditis or active Graves' disease).

4.15 Serious active infection requiring antibiotic therapy or other serious intercurrent illness. Patients with inflammatory bowel disease, Crohn's disease, ulcerative colitis, or active diverticulitis are excluded from study. Patients with active infections requiring antibiotic treatment (including chronic suppressive therapy) are not eligible until the infection has cleared and the antibiotics have been stopped for at least three days.

4.16 Prior radiation therapy to >50% of nodal groups.

4.17 Prior splenectomy.

4.18. Concurrent steroid use.

4.19 Patients with a history of seizures, encephalitis or multiple sclerosis are not eligible.

4.20 Patients who are pregnant, lactating or if of reproductive age, not using adequate contraception.

4.21 Patients who will be unable or unwilling to comply with the study protocol.

4.22 Patients who are seropositive for HIV or hepatitis B virus.

5.0 DRUG INFORMATION

The polynucleotide vaccine strategy employs the direct, intramuscular injection of naked plasmid DNA. The human CEA genes have been inserted into a simplified eukaryotic expression vector which utilizes the CMV major intermediate early promoter/enhancer to stimulate expression of the CEA. The plasmids are derived from the commercially available eucaryotic expression vector pcDNA3 (Invitrogen). The plasmid structure contains the cytomegalovirus early promoter/enhancer and the bovine growth hormone polyadenylation signal flanking a polylinker for insertion of heterologous open reading frames. The pcDNA3 plasmid has been modified by removal of sequences encoding the SV40 origin of replication and the neomycin resistance gene. Additionally, gene sequences encoding kanamycin resistance have been added. A detailed plasmid map is enclosed. The plasmid DNAs are grown in the E. Coli host strain IQ. Purification is by anion exchange chromatograpy utilizing Qiagen columns followed by extraction with Triton X-114 to remove endotoxin. The identity of the plasmid is verified by restriction endonuclease analysis. Purity of prepared DNA is validated by gel analysis, restriction endonuclease analysis and functional analysis for elicitation of immunity in a murine model of polynucleotide vaccination. Purified plasmid DNA for polynucleotide vaccination will be stored in lyophilized form in vials of 0.5 mg or 1.0 mg in sterile vacuoles. At the time of administration, the DNA will be resuspended in 1 ml sterile saline for injection and the appropriate volume administered at each dose level. The IND for this preparation will be obtained prior to trial initiation.

6.0 TREATMENT PLAN

6.1 Informed Consent

Prior to entry into the study, the protocol will be discussed with the patient and signed informed consent obtained.

6.2 Vaccination Strategy and Schedule

6.2.1 Overall Approach

Patients will be placed sequentially on study in groups of three patients with progressively increasing intensity of immunization. The decision to progress to the next dose level for patients receiving a single vaccination will be based on lack of acute toxicity following two weeks of follow-up and eight weeks of follow-up for the patients receiving three immunizations.

6.2.2 Treatment Schedule

Group #1 0.05 mg CEA polynucleotide vaccine into each deltoid muscle x 1 (Day 1)

#2 0.15 mg CEA polynucleotide vaccine into each deltoid muscle x 1 (Day 1)

#3 0.5 mg CEA polynucleotide vaccine into each deltoid muscle x 1 (Day 1)

#4 0.15 mg CEA polynucleotide vaccine into each deltoid muscle on Days 1, 22 and 43

#5 0.5 mg CEA polynucleotide vaccine into each deltoid muscle on Days 1, 22 and 43

6.3 Specific Therapeutic Plan

6.3.1 All vaccinations will be carried out in the out-patient department of the Clinical Research Center of the University of Alabama at Birmingham.

6.3.2 Each patient will receive bilateral intramuscular (deltoid muscle) injections of the CEA polynucleotide vaccine. The use of bilateral injections for each immunization is to reduce the likelihood that a technical failure of delivery into the body of the muscle will occur since such a delivery would preclude gene expression and immune response. Secondly, gene expression has been reported to be greater if more than one site is used (27).

The intramuscular injection technique will utilize standard aseptic technique utilizing a 1 ml syringe, 25 g needle and a volume of $\leq$0.5 ml for each injection. The patient will be monitored (vital signs Q 15 minutes times 4 and Q hour times 3) for four hours for local pain, discomfort or signs of inflammation and be re-examined 24 hours later to monitor for any local or systemic signs of inflammation or toxicity. The patient will be monitored by phone conversations at 48 and 72 hours and return for visits/exams weekly times 2 for evaluation for toxicity and blood samples as detailed later. Reporting of adverse reaction to the FDA and IRB will be strictly followed.

6.4 Order of Patient Entry

Three patients will enter the initial dose level treatment group and all patients will be monitored for at least two weeks before accrual to the next dose level can begin. The follow-up requirement for the multidose groups (Groups IV and V) will be eight weeks before moving to the next dose

level. Rules for need to expand patient accrual at any particular dose level or stop progression to the next dose level are described in section 7.1.

6.5 Management of Adverse Drug Reaction (also refer to section 5.5)

6.5.1 Acute Adverse Reaction to Polynucleotide Vaccine Administration

To date, no serious or even mild acute toxicities from polynucleotide vaccines have been seen in mice (25), rats (27), ferrets (36), or non-human primates (36). In our own research, over 100 mice have received intramuscular injections into the tongue on multiple occasions (up to 18 times/mouse) with doses of 0.01 to 0.25 mg without any evidence of subsequent pain, swelling or interference with grooming, eating or drinking.

Patients will be monitored for any local signs of inflammation and may receive local heat application and transient anti-inflammatory agents if symptomatic.

6.5.2 Late Toxicity

No late toxicities have been associated with polynucleotide immunization in preclinical studies. It is conceivable that the local expression of a protein with associated immune response could cause local inflammation and muscle fiber damage. This has not been seen to date and preclinical studies suggest that only a very few fibers actually express antigen so that their destruction would not be clinically significant. No toxicity was noted in mice receiving a polynucleotide vaccine of HBsAg despite a brisk immune response. The potential for CEA synthesis and release should have no adverse effects given that these patients will have metastatic colorectal cancer with varying amounts of CEA already present. Immune response to CEA could produce anti-tumor effects while reactivity to cross-reacting family members of CEA family could induce neutropenia, colitis, hepatitis, or other organ dysfunction. Such events have not been seen in preclinical studies in animals who have homologous CEA family members nor in humans (over 20) or non-human primates given recombinant vaccinia-CEA immunizations. Patients will be monitored for such adverse events and treated appropriately.

7.0 THERAPY MODIFICATION

7.1 Polynucleotide Vaccine

Local mild discomfort or aching at the site of injection will not alter dose or schedule decisions (Grade I) since this may simply reflect mild hematoma secondary to an intramuscular injection.

If any patient develops grade 2 toxicity of any kind thought to be related to the vaccine, the study will be interrupted, clinical course evaluated further and further patient accrual dependent on review and approval by the FDA. It should be noted that the patient population will have metastatic colorectal cancer with associated symptoms (pain, nausea, emesis, fever) and that disease progression can occur over a six to eight week period of follow-up.

If patients develop neutropenia, colitis or hepatitis, judged to be due to the vaccine and not to metastatic cancer, the study will be closed.

Groups I to III represent single vaccination schedules of increasing doses. Progression to the next dose level will require that no patient in the current dose level has a Grade II or greater toxicity over two weeks of follow-up.

Groups IV and V have repeat vaccine administrations and progression from Group IV to Group V will require that no patient in Group IV has a grade 2 toxicity secondary to the vaccine over eight weeks of follow-up.

7.2 Criteria for Removal from Study

7.2.1 Intercurrent illness which prevents further doses of the vaccine.

7.2.2 Development of clinically evident progression of disease.

7.2.3 Unacceptable toxicity.

7.2.4 Decision of the patient to withdraw from the study.

8.0 STUDY PARAMETERS

8.1 Schedule Group I-III

	DAYS								
	Pre[1]	1	2	8	15	22	29	43	57
History & Physical	x	x							x
Toxicity Evaluation (clinical)	x	x	x		x		x	x	x
Karnofsky Performance Status	x	x							x
Stage[2] (tumor measurements)	x								x
Polynucleotide Vaccine		x							
Vital Signs	x	x	x	x			x		x
CEA	x	x		x	x		x	x	x
CBC	x	x		x	x		x	x	x
SMAC, C-reactive Protein and Creatine Kinase	x	x		x	x		x	x	x
HIV	x								
HBsAg, HBsAb, HBcAb	x								
Pregnancy Test[3]	x								
Humoral Immune Response	x				x		x	x	x
Cellular Immune Response	x						x		x
Recall Ag Skin Test[4]	x								

[1]Within 30 days of starting treatment.
[2]Should include CT of the abdomen (pelvis, if indicated) and CXR.
[3]For all women of childbearing age.
[4]Skin testing with candida/trichophyton

8.0 STUDY PARAMETERS

8.1 Schedule

Group IV and V	Pre[1]	DAYS 1	2	8	15	22	23	29	36	43	44	50	57
History & Physical	x	x											
Toxicity Evaluation (clinical)		x	x	x	x	x	x	x	x	x	x	x	x
Karnofsky Performance Status	x												x
Stage[2] (tumor measurements)	x												x
Polynucleotide Vaccine		x				x				x			
Vital Signs	x	x	x	x		x	x	x		x	x	x	
CEA	x	x		x	x	x		x	x	x		x	x
CBC	x	x		x	x	x		x	x	x		x	x
Chem-18, C-reactive, Protein, Creatine Kinase	x	x		x		x		x		x			x
HIV	x												
HBsAg, HBsAb, HBcAb	x												
Pregnancy Test[3]	x												
Humoral Immune Response	x				x	x		x		x		x	x
Cellular Immune Response	x							x					x
Recall Ag Skin Test[4]	x												

[1]Within 30 days of starting treatment.
[2]Should include CT of the abdomen (pelvis, if indicated) and CXR.
[3]For all women of childbearing age.
[4]Skin testing with candida/trichophyton.

8.2 Evaluation During Study

Following vaccination, patients will be observed for local reaction at the injection sites as well as examination of the regional lymph nodes (Days 1, 2, 8, and 15). During each clinical toxicity evaluation (days 1, 2, 15, 29, 43, and 57) the NCI Common Toxicity Criteria form provided as Appendix A (Attachment 3) will be used as a checklist to identify and grade toxicity. Serial serum samples will be drawn to evaluate for an antibody response to CEA as well as CEA antigen. Blood for cellular immune studies will be obtained at Day 29 and 57 (four and eight weeks) and assayed for T cell mediated immune response to CEA. If any assay for cellular immunity is positive, a repeat study will be carried out within 4 weeks of the positive assay date.

8.3 Antibody Response to CEA

The Humoral Immunity Laboratory will carry out separate assays for antibody response to CEA. Each assay will be run with the pre-therapy and post-therapy serum samples on each patient on the same day to avoid inter-assay variability.

The assays are:

- solid phase ELISA using anti-IgG and anti-IgM detectors
- double antigen radiometric assay utilizing solid phase and ^{125}I-CEA detector

Sera with positive antibody assay results to CEA will be tested for ability to mediate ADCC and complement mediated lysis of CEA positive target cells. These assays are described in Appendix E.

8.4 Cellular Immune Response to CEA

The Cellular Immunology Laboratory will carry out assays for cellular immunity to CEA. The assays are:

- lymphoblastic transformation
- lymphokine release assay (IL-2)
- lymphokine release assay (IL-6)

8.5 Circulating Antigen Levels

Serum samples will be assayed for levels of CEA as indicated in section 8.1 using a Hybritech commercial radiometric assay kit.

9.0 EVALUATION CRITERIA

9.1 Adverse Drug Reaction Reporting Criteria

9.1.1 Report by telephone to FDA Division of Vaccines and Related Products within 24 hours (301-594-2090).

i) All life-threatening events (Grade IV) which may be due to drug administration.

ii) All fatal events.

iii) The first occurrence of grade 2 toxicity including previously unknown clinical event (regardless of grade).

9.1.2 Written report to follow within 10 working days to:

Food and Drug Administration
Division of Vaccines and Related Products
Dr. Alexander Kuda
1401 Rockville Parkway, Room 20010
Rockville, MD 20852-1448
301-594-5114 (phone)
301-594-1975 (fax)

9.1.3 Use the protocol number and IND number for this study on all reports.

9.1.4 **Grading of Toxicity** - Grades of toxicity are defined in Appendix A.

10.0 PATIENT REGISTRATION

All potential study candidates will be evaluated by the research nurse and the principal investigator. After confirmation of eligibility including a positive skin test to recall antigens, the patient will be registered and enrolled by calling the Clinical Studies Unit.

11.0 STATISTICAL CONSIDERATIONS

11.1 This is a phase Ia, non-randomized open label study to examine the toxicity of single and repetitive doses of a CEA polynucleotide vaccine. Patients will receive three single vaccinations with 0.1, 0.3 and 1.0 mg of vaccine. If no significant toxicity occurs (≥grade II) due to single vaccine administration, subsequent groups will receive 0.3 mg every 3 weeks times 3 and if no toxicity 1.0 mg every 3 weeks times 3. Patient groups will comprise 3 patients. Since no significant side effects should occur with this treatment strategy, the presence of grade II toxicity due to vaccine administration will stop accrual with subsequent consultation with FDA regarding proceeding with the study.

11.2 The immune response to these vaccinations will be assayed by both humoral and cellular immune assays. These results will be used as a guide for future phase I/II trials that will define optimal biologic dose/schedule using larger patient numbers for statistical significance.

11.3 The proposed accrual of 15 patients should be accomplished in a 1-year time frame.

Informed Consent

TITLE: Phase Ia Trial of a Polynucleotide Vaccine to Human Carcinoembryonic Antigen in Patients with Metastatic Colorectal Cancer

INTRODUCTION AND PURPOSE

I understand that I have metastatic cancer of the colon or rectum.. My cancer had spread outside the confines of the colon and is not surgically curable. I also understand that I have received chemotherapy with 5-fluorouracil (with or without other agents) and that my tumor has either not responded to therapy or relapsed after transient response.

Investigators at the University of Alabama at Birmingham Cancer Center are currently looking into innovative treatments to improve the therapy of metastatic colon and rectal cancer. I have been asked by my physicians to participate in a research study using a form of gene therapy tumor vaccine in an effort to stimulate the anti-tumor potential of my immune system.

This form of gene therapy utilizes the intramuscular injection of the gene for carcinoembryonic antigen (CEA), a tumor-associated antigen expressed on the surface of your colorectal cancer cells. It is expected that a small fraction of your muscle cells will express CEA and stimulate an immune response directed against your tumor cells. This form of gene therapy is called a polynucleotide vaccine since the gene injected into your muscle may induce an immune response to the CEA molecule. This polynucleotide vaccine to CEA antigen is investigational.

This gene therapy polynucleotide vaccine has been tested in animals and found to be safe and to induce anti-tumor effects. This type of vaccine therapy has not been given to patients previously so the side effects or toxicity are unknown. This trial will evaluate the side effects as well as the immunologic response to this polynucleotide vaccine.

EXPLANATION OF PROCEDURES

The initial groups of patients will each receive increasing doses of the vaccine dependent upon a lack of side effects in the prior group of patients. Later in the trial, patients will receive three vaccinations at three-week intervals using doses that were well tolerated as single vaccinations. A vaccination will involve an injection of vaccine into each deltoid muscle (back of arm) using a syringe and needle with a volume of less than 0.5 ml (less than 1/10th of a teaspoon). I will be closely followed for any side effects.

_____
Patient's Initials

Participation in this study will require that I have laboratory studies and x-rays including CT scan of the abdomen to assess the extent of my metastatic tumor. I will also have blood drawn for the various immunology studies. It may be necessary to draw up to one unit of blood (400 ml) monthly for two months for these special studies. My body should regenerate this blood within four weeks in the same fashion as would occur if I had donated blood. This therapy should not be given if I am pregnant and I will take measures to prevent pregnancy during the two study months.

The entire study will be conducted in the out-patient department and I will not be hospitalized unless problems – either due to the vaccination or otherwise – require admission to the hospital. The duration of the study will be eight weeks from initial vaccination.

RISKS AND DISCOMFORTS

This form of gene therapy and this particular polynucleotide vaccine have not been given to patients previously. However, studies in mice, rats, and monkeys have noted no toxicity or side effects associated with this type of vaccine administration. In animals, these vaccines have induced immune responses which are protective against challenge with infectious agents and some types of cancer cells. Possible side effects include those seen with vaccinations in general including local tenderness, discomfort or pain at the injection site, swelling and possible tenderness of regional lymph nodes (in arm pit). In addition to these side effects, it is theoretically possible that I could develop an immune response to CEA or CEA-like molecules in normal tissues which could cause unexpected toxicity to circulating white cells, lining of the colon or liver dysfunction. The likelihood of these risks cannot be identified because the vaccine is a new treatment. Animal studies and trials in humans with a different CEA vaccine have revealed no unexpected problems due to developing an immune response to CEA. There may be other risks which have not been encountered and cannot be anticipated.

Risks of having blood drawn include bleeding at the needle puncture site, leading to discoloration of the skin and possible iron deficiency. I will take an iron supplement during the trial.

The potential acute or long-term side effects of having a gene inserted into my muscle cells are unknown. The insertion of a gene into some of my muscle cells is considered harmless to me; however, events could possibly occur in muscle cells or other normal cells in the body that allow them to be cancerous. Laboratory studies suggest that this is highly unlikely and has not been observed in pre-clinical animal studies. However, this is a new procedure and we do not know whether cells could become abnormal after long periods of time. A second factor is that the vaccine also contains a gene that inactivates a certain antibiotic (kanamycin) in bacteria. This protein is not likely to be made in humans and many other antibiotics that are not inactivated by this protein are available and effective in treating potential bacterial infections. Finally, it is possible that this expression of the gene in my muscle cells could trigger an immune response that could lead to an autoimmune disease such as arthritis or inflammatory muscle disorders. Animal studies suggest that this is very unlikely.

_____
Patient's Initials

We emphasize that this procedure of administering genes directly into muscle cells is new and has not been done in humans previously. Because it is new and despite our extensive efforts to assure its safety, other unforeseen problems may arise including the very remote possibility that death could occur.

POTENTIAL BENEFITS

The therapy is not guaranteed to work, nor is it considered a cure. The possible benefit to me might be that my immune system is stimulated by the vaccine and develops anti-CEA immunity that can be monitored by special tests. Whether and how this effect translates to a clinical benefit is not yet known. The hope is that the development of anti-CEA immunity would be useful in my immune system's fight against CEA bearing colon cancer cells. Development of anti-CEA immunity might benefit me by causing tumor shrinkage with reduction of symptoms and prolongation of life. However, no such effects are known to occur. While I may not personally benefit from this research study, knowledge may be gained that will benefit others.

ALTERNATIVE TO PARTICIPATION

At the present time, there is no proven method to improve my survival and alternate chemotherapy programs have modest benefit. I may choose not to voluntarily participate in this study and be followed routinely by my physician. Alternatively, I could seek other alternate experimental therapy using investigational chemotherapy or immune therapy studies.

CONFIDENTIALITY

I understand that the results of my treatment, including laboratory tests and x-rays, may be published for scientific purposes. However, my identity is not revealed and confidentiality will be maintained as provided by state and federal law.

In addition, I understand that representatives from the Food and Drug Administration may monitor the trial records and that the individual conducting the review will see my name in the file folder. A qualified representative of the National Institutes of Health may inspect patient and study records. This procedure may attract the attention of the media. Every effort will be made to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning me and my treatment will appear publicly without my consent.

WITHDRAWAL WITHOUT PREJUDICE

I understand that my participation is voluntary and I am free to withdraw my consent and to discontinue participation in this project at any time without prejudice against future medical care I may receive at this institution. I also understand that my physician may discontinue this program if further treatment is felt to be dangerous or unwarranted. I understand I will be informed of any new findings that could influence my desire to continue with this treatment.

_____
Patient's Initials

PAYMENT FOR RESEARCH-RELATED INJURIES

The University of Alabama at Birmingham does not have provisions for monetary compensation in the event of physical injury resulting from the research, and in the event of such injury, medical treatment is provided, but is not provided free of charge.

COSTS

The investigational vaccine and its administration and follow-up doctor visits will not be charged to me or my insurance company. Special immunologic laboratory studies will also not be charged to me or my insurance company. Routine laboratory studies (complete blood counts, liver function studies, kidney function studies, blood levels of CEA, pregnancy test, tests for hepatitis B or human immune deficiency virus infection, etc.) as well as routine diagnostic studies to evaluate for the progression or regression of my disease (CT scans of the abdomen and chest x-rays) will be billed to my insurance carrier or will have to be borne by me. My physician and his staff will assist me in obtaining maximum third party coverage due me.

QUESTIONS

If I have any research-related questions or questions about the research compensation or treatment of research-related injuries, the following physicians will be glad to answer them: Albert F. LoBuglio, M.D. at (205) 934-5077 or Robert M. Conry, M.D. at (205) 934-5077. Also, I may contact the Senior Patient Representative, Ms. Tucker Slaughter, at (205) 934-2273 for questions concerning my rights as a research subject.

AGREEMENT

I have received a copy of this informed consent. I understand that I am not waiving any of my legal rights by signing this consent form. My signature below indicates that I agree to participate in this study.

_____	_____
Signature of Subject	Date
_____	_____
Signature of Physician	Date
_____	_____
Signature of Witness	Date

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
CURIEL, David T., M.D.	Associate Professor, Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
West Georgia College, Carrollton, GA	B.A.	1974-1978	Chemistry
Emory Univ. School of Medicine, Atlanta, GA	M.D.	1978-1982	Medicine
Emory Univ. Affiliated Hospital, Atlanta, GA	Intern	1982-1983	
	Resident	1983-1985	

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **DO NOT EXCEED TWO PAGES.**

1985-1989 Fellowship, Pulmonary Medicine, Pulmonary Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

1989-1990 Biotechnology, Navy Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

1990-1991 Visiting Assistant Professor of Medicine, Division of Pulmonary Diseases, Department of Medicine, The University of North Carolina, Chapel Hill, NC.

1991-1993 Assistant Professor of Medicine, Division of Pulmonary Diseases, Department of Medicine, The University of North Carolina, Chapel Hill, NC.

1992-1993 Curriculum in Genetics, The University of North Carolina, Chapel Hill, NC.

1992-1993 Member, UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

7/93-Present Associate Professor of Medicine, Division of Pulmonary Diseases; Director, Gene Therapy Program; Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham.

SELECTED PUBLICATIONS:

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Conry RM, LoBuglio AF, Kantor J, Schlom J, Loechel F, Moore SE, Sumerel LA, Barlow DL, Abrams S, Curiel DT: Immune response to a carcinoembryonic antigen polynucleotide vaccine. Cancer Res 54:1164-1168, 1994.

HUMAN GENE TRANSFER PROTOCOL

9406-074 Evans, C. H. and Robbins, Paul; University of Pittsburgh, Pittsburgh, Pennsylvania, *Clinical Trial to Assess the Safety, Feasibility, and Efficacy of Transferring a Potentially Anti-arthritic Cytokine Gene to Human Joints with Rheumatoid Arthritis.*

Date of RAC Approval: 6/9/1994

1. Scientific Abstract

Rheumatoid arthritis (RA) is a chronic, progressive disease thought to be of autoimmune origin. Although RA has systemic components the major pathologies occur in the joints, which suffer both inflammation and tissue destruction. These lead to loss of function and severe impediment, both economically and in terms of quality of life. Furthermore, patients with chronic severe RA have a shortened life expectancy. The prevalence of RA in the USA increases with age, affecting 5% of women aged 65 and older. It remains incurable and, in many cases, responds poorly to treatment. For many patients, the surgical removal of the diseased joint and its replacement by a prosthesis remains the only recourse.

In view of these severe deficiencies in the present treatment of RA, we have suggested a novel therapeutic strategy based upon the delivery of anti-arthritic genes to the synovial lining of joints. Expression of these genes leads to intraarticular production of their cognate proteins which, in the case of secreted proteins, are released into the joint. Thus the joint now becomes the site of synthesis of its own anti-arthritic proteins. Not only does this obviate the problem of delivering therapeutic proteins to joints, but also it minimizes exposure of non-target tissues and thus reduces side-effects.

Using the rabbit knee as a model, we have developed an *ex vivo* method for delivering genes to joints. A surgical, partial synovectomy is performed and the type B, fibroblastic synoviocytes cultured *in vitro*. A replication defective retrovirus, MFG, carrying the gene(s) of interest is used to transduce these cells. After confirming high *in vitro* expression of the transgene, the cells are autografted back to the knee joint by intraarticular injection. The injected cells recolonize the synovium and continue to produce the transgene product for several weeks. We have performed extensive studies with a cDNA encoding the human interleukin-1 receptor antagonist protein (IRAP) which has anti-arthritic potential; a phase II clinical trial using IRAP protein has recently been completed. Both human and animal data show that even very high levels of IRAP are completely non-toxic. Use of a MFG-IRAP vector in rabbits in conjunction with the *ex vivo* method alluded to above, results in the intraarticular accumulation of several ng per knee of biologically active human IRAP, which blocks the intraarticular pathologies that normally follow injection of interleukin-1. Preliminary data further suggest that delivery of the IRAP gene to synovium also inhibits the development of antigen-induced arthritis in rabbits. The *ex vivo* delivery method does not alter any of a battery of serum chemistries and hematological values that we have monitored in rabbits. Furthermore, we have not been able to detect any migration of the transplanted cells away from the knee joint into which they were injected.

In view of these highly encouraging data, we are now proposing a clinical trial in which the IRAP gene will be introduced into human, rheumatoid metacarpal phalangeal (MCP) joints by an analogous *ex vivo* approach. As this is the first time that a gene will have been transferred to a human joint, the overriding priority is that of safety. Because of this, several safety features are built into this study. The most compelling of these is that the gene will be introduced into MCP joints one week prior to their surgical removal during scheduled joint replacement surgery. However, as further security, a herpes simplex thymidine kinase gene will be co-transduced into the target cells, thus rendering them sensitive to ganciclovir. In this context it is also worth pointing out that extensive clinical tests of IRAP protein have found it to be devoid of toxicity.

Tissue recovered at the time of joint replacement surgery will be analyzed for the presence and expression of the transferred genes, and for evidence of a biological response to IRAP. Patients will undergo a rigorous, lifelong follow-up.

2. Non-technical Abstract

Rheumatoid Arthritis (RA) is a chronic, crippling disease which remains incurable and, in many cases, poorly treatable. The primary sites of disease are the joints where inflammation and tissue destruction lead to pain and disability. Chronic RA is also associated with a lower life expectancy. Because the medical control of RA remains inadequate joint replacement surgery remains the only reasonable option for many patients. We propose to break this stalemate by developing gene therapies for RA and other diseases of joints. Our basic strategy is to deliver therapeutic genes to a tissue known as the synovium, which lines all moveable joints. Once the genes are incorporated into the cells of the synovium (synoviocytes), these cells synthesize and secrete into the joint the anti-arthritic proteins encoded by the genes. In this manner, the joint now becomes the site of synthesis of its own anti-arthritic proteins.

For the past five years we have been developing methods for delivering genes to joints, using the rabbit knee as a model system. This joint is approximately the same size as the human knuckle joint to be used in this clinical trial. Synovial tissue is surgically removed from the rabbits' knee joints and the synoviocytes grown outside the animal where the genes of interest are then transferred to the cells using a harmless, modified virus. The genetically modified cells are injected back into the knee joints of the donor rabbits where they continue to produce the proteins encoded by the transferred genes for several weeks. We have failed to find any toxicity associated with this procedure.

We have devoted considerable attention to using a replication-defective retrovirus to deliver a gene encoding a potentially antiarthritic protein called the "interleukin-1 receptor antagonist protein" (IRAP). IRAP protein is presently in human phase II clinical trials for the treatment of arthritis, where it is proving to be non-toxic and of some benefit. We have successfully removed samples of synovium from rabbits' knees, grown the cells contained within these samples and used the virus to incorporate the IRAP gene into the chromosomes of the rabbit synoviocytes. These genetically modified cells are then injected back into the knees of the recipient rabbits, where IRAP production continues for several weeks. This process provides protection against arthritis in these knees.

We now propose to extend these methods to a human trial. Synovial tissue will be removed from human, rheumatoid joints at the time of scheduled joint replacement surgery. Cells will be grown outside the body in nutrient medium and infected with a modified retrovirus which not only carries the IRAP gene but also, as an additional safety measure, a gene encoding a viral protein known as "thymidine kinase" (tk). This renders the cells sensitive to the anti-viral agent ganciclovir, so that they may be easily eradicated if necessary. Modified cells will be injected into selected rheumatoid knuckle joints one week prior to normal, scheduled surgery to remove these joints and replace them with artificial joints. This provides a huge safety margin because the joints into which the genetically modified cells have been introduced will later be surgically removed during scheduled joint replacement procedures. Removal of the knuckle joints will remove all the experimental material, leaving the patient otherwise unaltered.

At the time of surgery tissue will be retrieved and analyzed for the presence and expression of the transferred genes, and evidence of a biological response to IRAP. Patients will be monitored periodically after surgery and their blood cells tested for the presence of viral sequences.

This trial represents a crucial first step towards the development of a safe, effective gene treatment for human RA.

CLINICAL PROTOCOL

Clinical Trial to Assess the Safety, Feasibility and Efficacy of Transferring a Potentially Anti-arthritic Cytokine Gene to Human Joints with Rheumatoid Arthritis

Principal Investigator:	Christopher H. Evans, Ph.D.
Co-Principal Investigator:	Paul D. Robbins, Ph.D.
Co-Investigators:	Joseph C. Glorioso, Ph.D. James H. Herndon, M.D. William B. Macaulay, M.D. Gunhild M. Mueller, Ph.D. Simon Watkins, Ph.D. Theresa L. Whiteside, Ph.D.
Consultants:	John A. Barranger, M.D., Ph.D. Michael T. Lotze, M.D.
Institution:	University of Pittsburgh

5. PROTOCOL

A. Rheumatoid Arthritis - The Clinical Problem

Arthritis is the most prevalent condition among those over 65 years of age (1,2), with over 30 million Americans suffering from the various arthritides. Of these, rheumatoid arthritis (RA) accounts for approximately 5 million individuals; around 150,000 new cases of RA are diagnosed each year in the USA (3). The prevalence of RA is greater in females than males, and increases with age; 5% of women aged 65 or more have RA. The lifetime costs of RA have been estimated as more than $29,000 per case in 1990 dollars (3). The cost in human suffering is impossible to quantify.

RA is a chronic, progressive inflammatory disorder which primarily affects joints. Associated with the disease is erosion of the soft tissues within the joint, particularly the articular cartilages. These pathophysiological changes lead to pain and functional failure of the joint. As such, RA is an agent of considerable human suffering which, if it affects the weight bearing joints, can lead to confinement to a wheelchair. Furthermore, patients with severe RA have a shortened life expectancy (4). Some of this is due to the toxicity of anti-arthritic drugs.

Despite a sizeable pharmacologic armamentarium, RA remains incurable. Most treatment protocols are based upon the traditional therapeutic pyramid (fig. 4). The first line of pharmacologic defense is usually administration of a non-steroidal antiinflammatory drug (NSAID); aspirin is one such drug. Although NSAIDs often produce some measure of symptomatic relief, they neither prevent loss of the articular cartilage, nor interfere with the underlying disease process. Furthermore, they have such high incidence of undesirable side effects, such as peptic ulcers, hypertension and hyperkalemia, that about 50% of patients taking any one NSAID discontinue its use within a year (5).

The next level of drugs contains the so-called "disease modifying anti-rheumatic drugs" (DMARDs), which include gold drugs, penicillamine and antimalarials (fig. 4). Although DMARDs are thought to slow the progression of RA, they are unpredictable in their effects and all have severe side effects. After 5 years of initiating DMARD therapy only 5-15% of patients persist with the drug (6). This leaves cytotoxic drugs and various experimental agents as the only remaining therapeutic options.

When all pharmacologic methods have been exhausted, the only recourse for the severely diseased, rheumatoid joint is the surgical insertion of a prosthetic replacement. A measure of the inability of existing drugs to combat RA may be gathered from the statistic that, in 1988, 190,000 artificial joints were implanted because of RA. This accounted for nearly 12% of all artificial joints inserted that year (7). Against such daunting statistics, the pursuit of novel therapies, such as gene therapy, would seem to be required.

These issues are discussed in Evans and Robbins (8), a copy of which is included in appendix D.

B. Rheumatoid Arthritis - Pathophysiology

The essential anatomy common to all diarthrodial (moveable) joints is shown in figure 5. Smooth articulation is ensured by the unique macromolecular structure of articular cartilage, which covers the ends of the bones. The articular cartilages move against each within a cavity, the joint space, which is lined by a tissue known as the synovium. In normal joints, the joint space contains a small volume of synovial fluid which is involved in joint lubrication and nutrition of the cartilage.

In normal joints the synovium is only 2-3 cell layers in depth. There are two types of synoviocytes: the type A cells which resemble macrophages, and the type B cells which are fibroblastic. It is the latter cells into which we will introduce genes as part of the present protocol. The synovium is underlain by a sparsely cellular sub-synovium, which, depending on the antatomical location, may be fibrous, adipose or areolar in nature. The anatomy and biology of the joint has been recently reviewed by Sledge (9).

Two pathological processes predominate in the rheumatoid joint - inflammation and tissue destruction. The inflammatory component is largely seen as a synovitis with associated increase in the volume and cellularity of the synovial fluid. In the inflamed synovium the type B cells undergo considerable hyperplasia (10). In addition, the resident type A and type B synoviocytes are joined by a variety of leukocytes, predominantly lymphocytes, macrophages and mast cells. Most of the lymphocytes are $CD4^+$ helper cells which are thought to be activated by as yet unidentified intraarticular autoantigens. The predominant antigen presenting cell is the macrophage. B-lymphocytes within the synovium secrete immunoglobulins, in particular rheumatoid factor, which can form immune complexes and activate complement. (For a review of these matters, see reference 11).

The major effector molecules in the inflamed joint are thought to be cytokines released from activated macrophages and synoviocytes (12-14). Analysis of rheumatoid synovial fluids reveals the presence of high levels of a number of their cytokines including interleukins-1,-6 and -8 (IL-1, IL-6, IL-8), tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor, transforming growth factor β and others (12-14). Of these, IL-1 and TNF-α have attracted the most attention because they not only induce joint inflammation but also trigger loss of articular cartilage (15-17). Although a variety of pro-inflammatory cytokines exist in rheumatoid joints, IL-1 and TNF-α are the only well characterized cytokines known to cause cartilage loss. They do this in two ways. Firstly, they provoke chondrocyte-mediated breakdown of the extracellular matrix of cartilage; secondly, they suppress the synthesis of new matrix (18-20).

Most new strategies to improve the treatment of RA are based upon manipulating

the interactions between T-cell receptors and MHC-class II molecules present on the surfaces of antigen-presenting cells, or inhibiting the activities of IL-1 or TNF-α. The present application clearly falls into the latter category. We have preferred IL-1 over TNF-α because it is a stronger inducer of cartilage breakdown (19) and because there exists a safe, natural antagonist of IL-1, the interleukin-1 receptor antagonist protein (IL-1ra or IRAP), which has already completed phase II clinical trials without showing any toxicity. The properties of IRAP are discussed in the following section.

C. The Interleukin-1 Receptor Antagonist

The interleukin-1 receptor antagonist protein is a naturally occuring inhibitor of the biological actions of both IL-1α and IL-1β. Originally identified as a product of macrophages, several additional cell types have been shown to produce this molecule. An extensive review has been published recently by Arend (21).

Secreted human IRAP is a glycoprotein, whose core protein has a M.W. of approximately 17,000 Da. Addition of N-linked polysaccharides produces mature molecules of 22-25 kDa. IRAP binds to the type I IL-1 receptor with approximately the same affinity as IL-1α and IL-1β but, because it lacks agonist activity, acts as an inhibitor of cellular responses to IL-1 (22). Because of the involvement of IL-1 in many inflammatory diseases, IRAP has considerable therapeutic potential and has shown efficacy in a variety of animal models of disease, including arthritis (23-25). A human phase II human trial of IRAP in human arthritis has just been completed. Preliminary data from the trial have been presented in abstract form (26) but a full length account has not yet appeared. In this trial, patients self-administered IRAP by daily, subcutaneous injection at 0.5, 1, 2, 4 or 6mg/kg. By this method, mean plasma concentrations of IRAP were 39 +/- 36ng/ml at 0.5mg/kg and 600 +/- 240ng/ml at 6mg/kg 24 hours after injection. IRAP did not accumulate with daily injection. At 6mg/kg the mean number of tender joints decreased, erythrocyte sedimentation rates improved, and c-reactive protein levels dropped. These preliminary data suggest that IRAP has potential as an anti-arthritic protein, but is difficult to deliver as a drug. Daily self-injection at high doses is clearly not an acceptable option for a chronic disease such as RA. It is precisely for these reasons that we suggest gene delivery (24).

D. Gene Therapy for Rheumatoid Arthritis

Our group first suggested the use of gene therapy for RA in 1989 (27). This was based upon the realization that, although several novel proteins held great potential for use in treating arthritic joints, there was no satisfactory way to deliver them to the intraarticular site of disease. Because proteins cannot be taken orally, or delivered transdermally, and because proteins are, in general, metabolically labile, they would need to be injected into patients at regular intervals. Furthermore, because proteins are not freely diffusible into joints, they would need to be injected at very high doses. The diffusibility problem can be obviated by direct, intraarticular (i.a.) injection but, ironically, proteins are cleared from joints very rapidly via the lymph. Besides, repeated i.a. injection is not feasible.

To address these concerns we have been developing ways to deliver to the synovial lining of joints genes which encode potentially therapeutic proteins (fig. 6). When successful, cells within the synovium now synthesize the proteins of interest which, in the case of secreted proteins, accumulate intraarticularly, that is at the site of disease. In this way, the joint now becomes the site of synthesis of its own therapeutic agent. Thus in the sense on which we are applying it, gene therapy is being used as a drug delivery system (8,24,27). Depending upon the turnover of cells within the synovium, the persistence of the transferred gene and promoter activity, there is the potential for prolonged expression of the therapeutic DNA.

Genes may be delivered to synovium by *in vivo* or *ex vivo* approaches (fig. 7). Our group is actively investigating both of these. However, at the present state of development of the field, with safety as a paramount consideration, we strongly favor the *ex vivo* method for human trials. There are several reasons for this. With the *ex vivo* approach no viral particles are introduced into the joint. This means that there can be no sensitization of the patient to viral protein. In addition, it gives the investigator full control over the cell type which becomes transduced. Injection of vector directly into the joint risks the transduction of lymphocytes as they traffic through the joint and out again to other parts of the body. The long-term effects of transgene expression at untargeted organs are unknown. Furthermore, with the *ex vivo* method, all genetic manipulations occur outside the body and thus permit all and any required tests of the transduced cells to be carried out prior to their reintroduction into the body.

As described in the following section, we have used the rabbit knee as a model system for developing an *ex vivo* method for delivering genes to the synovial lining of joints (28,29). It involves the surgical removal of synovium from the donor, its growth in nutrient medium *in vitro* and its transduction with a replication-defective retrovirus known as MFG. An almost identical virus has already been approved by the RAC committee for use in *ex vivo* gene therapy for cancer (Somatix group) and for Gaucher disease (P.I.-J.A. Barranger). The principal investigator on the latter protocol is a consultant to the present one. One of the co-investigators of the present protocol (Paul D.Robbins, Ph.D.) developed MFG in the laboratory of Dr. R. Mulligan of the Whitehead Institute.

In the present protocol MFG will be used to carry into human synovial fibroblasts (type B synoviocytes) two genes. One codes for IRAP and the other for a so-called "suicide" gene, herpes simplex thymidine kinase C which renders cells expressing it sensitive to the antibiotic ganciclovir. This is used as an additional safety measure (q.v.). By convention, an MFG virus carrying two transgenes is called DFG. Thus the vector we propose to use in our clinical trial is designated DFG-IRAP-tk.

E. Proposed Clinical Study

Flow diagrams outlining the proposed clinical trial are shown in figures 8 and 9.

Patients who will be recruited for this study will only be those with a clinical indication for the staged removal of synovium from multiple diarthrodial joint(s) including 2nd-5th MCP joint arthroplasty. For example, the patient who eventually receives the 2nd-5th MCP joint arthroplasty may have a clinical indication for ipsilateral and/or contralateral therapeutic wrist synovectomy, or total elbow, knee, or hip arthroplasty at which time ample amounts of synovium (in addition to what might be sent for histopathological analysis) will be obtained and sent to the laboratory for culture and genetic modification. Since this procedure would have been performed anyway, the patient has not yet been affected by participating in this study.

At an appropriate time, probably 6 weeks to 3 months after the synovium has been collected, reimplantation of the modified cells will occur. Synoviocytes which have been successfully transduced with DFG-IRAP-tk, and which show high expression of IRAP and sensitivity to ganciclovir *in vitro*, will be returned by intraarticular injection to 2 out of 4 of the patients' MCP joints scheduled for joint replacement. The other 2 MCP joints will receive back untransduced cells as controls. The study subject will visit Dr. Herndon's office one week prior to the scheduled 2nd-5th MCP joint arthroplasty. During this visit, the patient's autologous modified synoviocytes will be returned by intraarticular injection into the MCP joints. Two MCP joints will receive cells transduced with the IRAP and tk genes. The other two MCP joints will serve as controls and receive unmodified cells. Prior to reimplantation, cells will be harvested by trypsinizing, thoroughly washed in saline solution, and resuspended at a concentration of $2x10^7$ cells per ml in saline solution.

At the time of reimplantation, each MCP joint will first be lavaged with 1ml of saline solution. If the intraarticular injection is excessively distressing, dorsal radial and dorsal ulnar nerve block will be performed using local anesthetic. In-office injections of any sort will be performed under scrupulously sterile conditions. After lavage, the barrel of the syringe will be removed leaving the 27G needle in place. A fresh barrel containing 0.5ml of the appropriate cell suspension will then be attached to the needle, permitting intraarticular injection of the cells. Aside from follow-up blood drawing described below, this will be the only extra procedure that patients will undergo as a result of this study.

As scheduled, the patient will undergo 2nd-5th MCP joint arthroplasty of those joints which had been manipulated one week previously. During this procedure, the joints will again be lavaged with 1ml of saline. Synovium/sub-synovium/adjacent joint capsule will be surgically removed and placed in their respective, clearly labeled, sterile containers for transport to the appropriate laboratory. The 2nd-5th MCP joint arthroplasty procedure will be approached in a manner identical to that used for tumor cases. Caution will be taken to thwart contamination of the surrounding tissues with joint contents. All OR personnel and couriers will be properly advised to the significance of the samples being sent to the laboratory. Solid tissues will be sent in sterile saline promptly to the laboratory.

The lavage fluids collected in the OR and "baseline" lavages obtained one week earlier will be analyzed for evidence of transgene expression and for evidence that transgene expression has altered various clinical parameters (Fig. 9). IRAP concentrations will be measured by conventional ELISA, and the presence of IRAP protein of the correct size will be further confirmed by Western blotting. To assess whether IRAP has altered pathophysiological processes within the joint, we will measure leukocyte concentrations, and additionally measure levels of IL-1β, IL-6, IL-8 and PGE_2. All of these are induced by IL-1 and are thus down-regulated by IRAP.

Solid tissue (synovium, sub-synovium and adjacent capsule) will be divided into three samples. One will be cut into frozen sections which will be used for *in situ* hybridization to detect the presence of tk message, immunohistochemistry to detect the presence of tk protein and standard hematoxylin and eosin staining for histological examination. A second sample will be used for extraction of DNA and mRNA for detecting the presence of DFG-IRAP-tk genes by PCR, and DFG-IRAP-tk mRNA by RT-PCR. A third sample will be placed into organ culture to detect the ganciclovir-sensitive production of recombinant IRAP. If insufficient material is available, the first two sets of assays will be given priority.

Each patient will undergo complete history and physical examination (H&Ps) at 2nd-5th joint arthroplasty, and at 2 weeks, 6 weeks, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 7 years, 9 years and every two years thereafter. At the 2nd-5th MCP joint arthroplasty and 2 weeks, 6 weeks, 3 months, and 1 year visits for follow-up, 25ml of blood will be drawn and the leukocytes analyzed for the presence of viral DNA by PCR. Serial H&Ps and blood draws comprise the extent of patient monitoring for this study.

The remainder of the clinical procedures which might be utilized for this study relate to the possibility, though unlikely occurrence, of untoward events. The most likely complication of participation in this study would be septic MCP joint induced at the time of genetically modified synoviocyte reimplantation. This complication would be clinically dealt with in an appropriate fashion on a case-by-case basis depending upon extent, location and identity of inciting pathogen. Appropriate antibiotic therapy would be initiated immediately. Open drainage of the affected joint(s) and temporary delay of the 2nd-5th MCP joint arthroplasty would comprise a worst case scenario.

In rabbit studies, we have failed to detect migration of the transplanted cells from the joint into which they were injected. However, as an additional safety measure, the tk gene has been included in the vector. In the highly unlikely event that eradication of the transduced cells were necessary, ganciclovir would most likely be administered over one hour intravenously at a dosage of 5mg/kg every 12 hours for 14 days in a patient with normal kidney function. The dosage and response to therapy would be evaluated by the infectious disease consultant.

The generation of replication competent virus poses a theoretical risk. However, the viral supernatents will have been analyzed for this prior to use, and the presence of helper virus will be assayed after infection of the synovial cells and prior to transfer.

So far as we know, this is the first proposal to conduct a clinical trial for the development of a gene therapy for a chronic non-lethal disease. This means that very strict safety measures have to be built into the study. We have done this in the following ways:

1. Joints receiving the genetically altered cells will be surgically removed one week after introduction of the cells. Our rabbit studies (see next section) have failed to detect the escape of transplanted cells from the joint into which they were injected for as long as six weeks. Furthermore, no changes in haemotopoietic parameters or blood chemistries occur during this time.

2. IRAP occurs naturally in human synovial fluids and elsewhere in the body.

3. Extensive toxicological testing of IRAP protein in animals, as well as pharmacologic and clinical testing in humans, has failed to reveal any evidence of adverse reactions.

4. We will incorporate a "suicide" gene, thymidine kinase, into the vector to render transduced cells sensitive to ganciclovir.

Patient discomfort has also been minimized. Those eligible for the trial will have polyarticular disease requiring staged replacement of other joints, such as the wrist, prior to MCP replacement. Synovial tissue will be recovered at the time of these prior surgeries. Synoviocytes will be cultured, transduced and tested while the patient is recovering from the initial joint replacement surgery and preparing for MCP replacement. Testing of cells for replicaiton competent virus, and adventitious agents will be conducted by Microbilogical Associates.

One week prior to scheduled MCP surgery, MCP joints will be lavaged and the cells injected. This is the only extra procedure that patients will undergo. It is relatively minor. MCP replacement surgery then proceeds as planned, with analysis of the retrieved tissue proceeding as described elsewhere in this protocol.

F. PRE-CLINICAL STUDIES

(i) Vector Construction

MFG Vector: The MFG vector is derived from MoMLV and has been described previously. The nucleotide numbers given for retriction sites in MoMLV are based on a numbering system where nucleotide 1 is the first base in the R region of the 5' LTR and thus the first base in the transcribed RNA. The MFG vector contains gag sequences up

to the Nar1 site at position 1035 to increase the packaging efficiency of the vector. The vector also contains a 390 base pair MLV fragment from the Nde1 (5402) to Xba1 (5794) sites which contains the splice acceptor. An 18 base pair oligonucleotide containing Nco1 and BamH1 sites directly follows the synthetic oligonucleotide which allows for insertion of cDNAs with compatible ends. The Nco1 site is positioned so that the initiation codon of the inserted gene is fused to the initiation ATG of the viral *env* gene. Thus the inserted gene is expressed from a spliced message that resembles the normal *env* message of MoMLV. The MoMLV LTR drives expression of the mRNA which contains a 5' untranslated region identical to the normal env message followed directly by the coding region of the inserted gene. In the MFG vector, there is an 8 base pair SacII linker inserted just downstream of the gag ATG at a HaeIII site which shifts the gag reading frame out of frame.

Construction of the MFG vector: The retroviral vector was constructed in several steps. First a three part ligation was performed where a Xho1 (position 1035; originally a Nar1 site) to Nde1 (position 2295 in pBR322) 5' LTR fragment from a half gag vector, and a BamH1 (originally a Cla1 site at position 7675) to Nde1 (position 2295 in pBR322) 3' LTR fragment from the pEM vector were ligated to a Xho1 to BamH1 histone promoter filler fragment. Second, the Nde1 site (position 2295 in pBR322) was destroyed by partial digestion with Nde1 followed by a klenow fill in reaction. The Xho1 site (originally a Nar1 site at position 1035 in MoMLV) was Nde1 linkered. Third, the large Nde1 to BamH1 fragment from the intermediate vector was then ligated to a Nde1 (position 5402 in MoMLV) to Xba1 (5794) fragment from MOV-9, containing the splice acceptor sequence, and a synthetic adapter fragment containing a Nco1 site positioned so that the ATG within the Nco1 recognition site is the initiation codon of the env gene.

Adapter sequence: C<u>ATG</u>ACTGCCATGGCGCG
TGACGGTACCGCGCCTAG

Cloning of IRAP: A human monocyte cDNA library in lambda gt10 was purchase from Clontech (catalog number HL1036a). The library was derived from the human monocyte cell line U937 which had been stimulated with 10 nM phorbol 12-myristate 13-acetate for 48 hours before isolation of the mRNA. The library was screened for IRAP clones via a standard plaque lift assays from agar plates to nitrocellulose filters. Two phage clones with EcoR1-flanking sites were isolated and subcloned into the EcoR1 sites of pUC18 and shown to have identical restriction maps. One of the clones was then sequenced and amplified by PCR. The resulting IRAP insert had the following structure: a 5' HindIII site followed immediately by bp 1, the entire IRAP protein coding sequence, 3' flanking DNA from bp 543-577, followed immediately by a 3' HindIII site. This insert was subcloned in the HindIII site of pSV2.

Construction of MFG-IRAP: The cDNA for human IRAP was inserted into the retroviral vector MFG by first BamH1 linkering the 3' HindIII site downstream of the stop coding in the IRAP gene followed by digestion with Pst1 and BamH1. The

Pst1-BamH1 IRAP fragment was then inserted into MFG, digested with Nco1 and BamH1, using a synthetic adapter CATGGAAATCTGCA
CTTTAG
containing a 5' Nco1 site and a 3' Pst1 site; the initiation codon of IRAP is underlined. The resulting plasmid, MFG-IRAP (see Fig. 2) contains the entire coding sequence of IRAP.

Construction of MFG-TK: To introduce the herpes simplex tk gene into MFG, 5' Nco1 and 3' BamH1 sites were introduced into the tk gene by PCR. A 5' primer was used that converted the two base pairs immediately 5' to the ATG of tk to CC, resulting in a Nco1 site. The 3' oligonucleotide pirmer was used that converted a single A nucleotide to a T nucleotide 29 bases downstream from the stop codon of tk to create a BamH1 site. The PCR reaction was performed using Vent polymerase. The resulting 1151 base pair fragment was digested with Nco1 and BamH1 and inserted into MFG at the Nco1 and BamH1 sites to give MFG-tk (see Fig. 3).

Construction of DFG-IRAP-tk: MFG-tk was digested with Nco1 and DraIII and the large fragment isolated. DraIII digests MFG at a site 172 base pairs upstream from the Nco1 site. The pCITE plasmid (Novagen) containing the encephalomyocarditis virus internal ribosome entry site (pCITE: Novagen) was digested with EcoR1, BamH1 linkered, and then digested with BamH1 and Nco1. MFG-IRAP was digested with DraIII and BamH1 and the 737 base pair IRAP-containing fragment isolated. The MFG-tk fragment, IRAP and the 600 base pair IRES BamH1 to Nco1 fragment were ligated in a three part ligation to give DFG-IRAP-tk (see Fig. 1).

Generation of high titer producer lines: The CRIP packaging line used for the experiments was checked by Microbiologic Associates for the presence of certain contaminating biological agents prior to use. The MFG-IRAP and MFG-tk vectors were cotransfected into CRIP packaging cells to generate high titer producers. 10g of the vector was cotransfected with 0.5 g of pSV2neo and the cells selected in G418 (.75 mg/ml). Single colonies were isolated and expanded. The supernatants from the clones were screened for high titer virus. For MFG-IRAP, two mls of supernatant from each of the expanded colonies was added with 8μg of polybrene/ml to a 10 cm2 plate of NIH 3T3 cells approximately 50% confluent. 72 hours post infection the supernatants from the infected cells were assayed for IRAP production using an ELISA assay (R&D Systems). The producer able to confer the highest level of IRAP expression to 3T3 cells 500ng/10^6 cells/day was then assayed for presence of replication competent virus as described below. For MFG-tK, 2 mls of supernatant from each of the expanded, G418 resistant colonies was added to 143tk- cells with 8 g/ml of polybrene. 48 hours post-infection the infected cells were split 1:10 into media containing HAT media and the number of tk+ colonies determined two weeks post-infection. The highest producer ($3x10^5$/ml) was then checked for replication competent virus. A similar method is currently being used to generate a high titer DFG-IRAP-tk producer. If the titer of DFG-IRAP-tk needs to be increased, multiple rounds of infection of the CRIP-DFG

producer will be performed using an ecotropic DFG producer. A similar method has resulted in an enhancement of the titers of many of the MFG-based vectors without the detection of replication-competent virus. The best producer of DFG-IRAP-tk as determined by IRAP expression and tk resistant colonies will also be titered by Southern analysis to confirm the integrity of the provirus structure after infection.

Detection of replication competent virus: To determine if the producer lines contained replication competent virus, a BAG mobilization assay was, and will be, used. Briefly, we have established a 3T3 population infected with the BAG retrovirus, expressing both LacZ and neor, and G418 selected. Supernatant from the CRIP producer was added to the 3T3-BAG cells and cocultured through several passages in the presence of polybrene. Supernatants from these cocultures were used to infect 3T3 target cells and the infected cells selected in G418. As a positive control, supernatant from a PA317 producer which had a low level of helper virus was used. The MFG-IRAP and MFG-tk producers were unable to mobilize BAG: no G418 resistant colonies were detected.

Expression of two genes in MFG (DFG vector): The strategy employed to express IRAP and tk genes in the same MFG vector is to use an internal ribosome entry site in the context of a polycistronic message. We previously have demonstrated that two genes can be expressed efficiently in MFG using a polycistronic message with one, and even two, IRES sequences. To examine the level of expression of two genes in MFG, IRAP and human growth hormone were used initially since their expression is easily assayed in a quantitative manner by ELISA and RIA respectively. A restriction map of DFG-IRAP-GH is shown in fig. 10. The level of IRAP and growth hormone expression from the DFG-IRAP-GH vector was compared to the level of expression of each protein from MFG-IRAP and MFG-GH vectors. BOSC23 cells were transiently transfected with the three plasmids and the supernatants harvested 48-72 hours post-transfection. 3T3 cells were infected with 5 ml of the BOSC23 supernatants in the presence of 8 g of polybrene. 72 hours post-infection the level of growth hormone and IRAP was determined in the media of the infected cells. As is shown in table 1, the level of expression of the two genes in the DFG vector were roughly equivalent to their levels of expression in the single MFG vectors. However, it appears as if expression of the gene 3' to the IRES is expressed at a slightly lower level. Therefore, the vector carrying the HSV tk gene were constructed with the tk gene 3' to the IRES sequence.

(ii) *Ex Vivo* Gene Transfer to Rabbit Knee Joint

New Zealand White rabbits weighing 5-6lbs are anesthetized by i.v. injection of 1.5ml of Nembutal, one knee joint shaved and a partial synovectomy performed by sharp dissection. Cells released from the synovial sample by digestion with clostridial collagenase (0.2% w/v; 3h; 37°) and seeded into 25cm^2 plastic culture flasks in the presence of 4mls of Ham's F_{12} medium containing 10% fetal bovine serum. When confluent, the cells are recovered by treatment with trypsin (0.25% w/v) and seeded at a 1:2 split ratio into further flasks. After 3 such passages any monocytes, macrophages,

mast cells, endothelial cells, type A synoviocytes or lymphocytes present in the original tissue are lost and the cultures are uniformly fibroblastic.

When 3rd passage cells are approximately 75% confluent, they are infected with MFG-IRAP. To do this, media are removed, and replaced by 1ml of viral suspension (10^6 infectious particles) in the presence of 8μg/ml Polybrene. After a 2h incubation with intermittent shaking, an additional 3ml of medium is added and cultures returned to the incubator for 72h. Cultures were then trypsinized and reseeded at a 1:5 split ration.

Media conditioned by the transduced cells are then tested for human IRAP both by a commercial ELISA assay and by Western blotting. Up to 500ng/10^6 cells/3 days of IRAP are produced by the cells. The protein generally runs as a single, glycosylated band corresponding to a molecular weight of approximately 25 kDa (Fig. 8). Sometimes a doublet is seen, with bands of approximately 22 and 25 kDa (not shown).

Having confirmed high expression of the transgene, 10^7 cells transduced are injected back to one or other of the transduced knee joints of the donor rabbit. (Repeated experiments have shown that it doesn't matter which knee is used). Combined histological and immunohistochemical staining of the transplanted cells suggest that they are captured by the synovium. Some of the cells insinuate themselves into the synovial interstitium, while other remain attached to the synovial surface where they appear to surround themselves by a collagenous capsule. Either way, the object of the transplantation has been achieved, with the transplanted cells remaining associated with the synovium and secreting IRAP (30).

Serial lavage of the joint space with 1ml of saline followed by ELISA assay permits assessment of *in vivo* expression of IRAP. As shown in fig. 12, one week after transplant there are several ng/knee of IRAP, an amount which declines progressively with time such that by 5 weeks, the values are close to the limit of detection of the assay.

The IRAP produced intraarticularly by this *ex vivo* method is biologically active and able to suppress the pathological changes that follow a single intraarticular injection of human IL-1β. These changes include the influx of leukocytes into the joint space, synovial thickening and inflammation, and release of glycosaminoglycans from the cartilaginous matrix (28,29 - see reprints in appendix D).

(iii) Efficacy in Antigen-Induced Arthritis in Rabbits knee

Experiments are underway to evaluate the efficacy of *ex vivo* IRAP gene transfer in antigen-induced arthritis in the rabbit knee. In this model, animals are pre-sensitized to ovalbumin by intradermal injection, and then given an intraarticular injection of ovalbumin (100μg) in one knee joint. A severe, erosive arthritis ensues which has an acute phase of approximately 1 week, superseded by a chronic phase which can last several weeks. We have begun by testing the ability of the IRAP gene to suppress the acute phase of the disease.

The experimental design is shown in figure 13, with arthritis initiated on day 1, the autologous cells, with or without the IRAP gene, transplanted on day 2 and the animals euthanized on day 4. Lavages are tested for human IRAP and GAGs, and the number of leukocytes counted. Cartilage matrix synthesis is measured by the covalent incorporation of $^{35}SO_4^{2-}$ into proteoglycan, and the synovium is evaluated histologically.

Initial results show that IRAP expression is higher in arthritic knees than non-arthritic knees (fig. 14), and that measures of cartilage breakdown (fig. 15) and suppression of cartilage synthesis (fig. 16) are normalized by IRAP. Leukocytosis is also suppressed by the IRAP gene but to a lesser degree (fig. 17). These data are consistent with the literature suggesting that IL-1 is the predominant mediator of cartilage loss, but only one mediator among several other, of inflammation. The observation that IRAP expression is higher in arthritic knees is an encouraging, unexpected result of obvious utility in the present context.

(iv) Safety Studies

Possible adverse effects of these procedures have been monitored in New Zealand white rabbits. Two safety aspects have received particular attention. First: Do the transplanted cells migrate from the joint into which they were injected? Second: Is there any change inhematologic parameters or blood chemistry?

Synovia were obtained from 27 rabbits by surgical partial synovectomy carried out under anesthesia, using a medial approach. A retroviral vector, BAG, carrying two marker genes, lac Z and neo, was used to transduce the synoviocytes grown from these samples. Autologous neo-selected cells (10^7 cells suspended in 1ml Gey's balanced salt solution) were injected into the left knee of each rabbit. At 1, 2 or 6 weeks post-transplant, 7mls of blood was withdrawn from the ear vein prior to euthanasia.

Blood samples were analyzed for the hematological and chemical parameters listed in table 2. Two-tailed t-tests have been performed for all values, and fail to show a statistically significant difference between control and cell-injected rabbits at any time.

After euthanasia, tissue was taken from the injected, left knee, the uninjected, right knee, liver, lung, heart and spleen. Samples of each tissue were treated with collagenase and then recovered cells grown *in vitro* under neo-selective conditions. Any neo^+ cells were stained with X-gal for expression of the lac Z gene. As shown in Table 3, neo^+, lac Z^+ cells were only recovered from the site of injection. No such cells could be recovered from the contralateral knee, liver, lung, heart or spleen.

Further samples of each organ are presently being examined by PCR for the presence of lac Z gene sequences.

(v) Transduction of Human Synoviocytes

Our laboratory routinely grows synovial fibroblasts obtained from human, rheumatoid synovia, and are have shown these to be susceptible to transduction by MFG-IRAP.

In conjunction with Theresa Whiteside, Ph.D., director of the Adoptive Immuno Therapy Laboratory, and a co-investigator on this proposal, we are optimizing the conditions for culturing human synoviocytes grown in defined medium (Basal Fibroblast Growth Medium, Clonetics) supplemented with human type AB serum, and for transducing them with MFG-IRAP-tk.

(vi) Human Synovial Fluid Analysis

Malyak et al 1993 (31) recently reported mean levels of 17.1 +/- 12.6ng/ml IRAP in 13 human rheumatoid synovial fluids. Fluids from three other RA patients had no detectable IRAP. In a similar study, Miller et al (32) reported synovial fluid IRAP levels in Lyme arthritis between 0 and 26ng/ml.

We have also analyzed a series of human rheumatoid and non-rheumatoid synovial fluids for the presence of IRAP. In agreement with the literature (31,32), we have also found detectable IRAP concentrations. Initially, we feared that this would compromise our ability to measure expression of transgene expression in human rheumatoid fluids. However, Western blotting has repeatedly shown that the IRAP present in human rheumatoid fluids migrates analogously on SDS-PAGE, appearing as a band of approximately 50 kDa in M.W. (fig. 18). The transgene product, however, runs authentically at approximately 25 kDa. When rheumatoid synovial fluid is mixed with transgene IRAP, a Western blot reveals two bands, one at 50 kDa and the other at 25 kDa, confirming the authenticity of this distinction (fig. 18).

Preliminary data using a bioassay based upon the mitogenic response of a mouse thymocyte cell line, D.10 to IL-1, suggest that the high M.W. IRAP present in rheumatoid fluid has reduced bioactivity. This would explain why the relatively small increment in IRAP concentrations produced in the human trial (see section 6-C, p. 21) produced a measurable clinical effect. It also permits optimism that the IRAP of the correct M.W. synthesized by DFG-IRAP-tk infected cells will produce a measurable biological effect.

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* - copies of these references are included as Appendix D

University of Pittsburgh
Medical Center

Musculoskeletal Institute

986 Scaife Hall
Pittsburgh, PA 15261
412 648 1090/648-1092
Fax 412-648 9113

Musculoskeletal Research Center
Christopher H. Evans, PhD
Professor
Department of Orthopaedic Surgery
Department of Molecular Genetics and Biochemistry
Director
Ferguson Laboratory

Approved by Biomedical IRB
University of Pittsburgh on:

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Consent to Act as a Subject in an Experimental Study
Competent Adults (18 years of age or older)

TITLE: **Clinical trial to assess the safety, feasibility and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis**

INVESTIGATORS: **C.H. Evans, Ph.D.**
Professor, Department of Orthopaedic Surgery and Molecular Genetics and Biochemistry
University of Pittsburgh School of Medicine
Principal Investigator

James H. Herndon, M.D., M.B.A.
Professor and Chairman, Department Ortho. Surgery
University of Pittsburgh Medical Center
University of Pittsburgh School of Medicine
Co-Investigator

Paul D. Robbins, Ph.D.
Assistant Professor, Department of Molecular Genetics & Biochemistry
University of Pittsburgh School of Medicine
Co-Investigator

Joseph C. Glorioso, Ph.D.
Professor and Chairman, Department, Molecular Genetics & Biochemistry
University of Pittsburgh School of Medicine
Co-Investigator

William B. Macaulay, M.D.
Resident, Department of Orthopaedic Surgery
University of Pittsburgh Medical Center
University of Pittsburgh School of Medicine
Co-Investigator

SOURCE OF SUPPORT: **National Institutes of Health**
University Orthopaedics, Inc.
Pittsburgh Genetics Institute

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Patient Initials

DESCRIPTION

This study is being done to see if gene therapy can be used as a treatment for rheumatoid arthritis. We will not cure rheumatoid arthritis in your joints which we study. We hope that this may be an important step towards a cure for rheumatoid arthritis and other joint diseases. The specific purpose of this study is to see if we can provide painful joints with modified joint cavity lining cells which make more of a natural human joint substance (a protein) which has been shown to decrease joint swelling. We have shown already that when we put an anti-swelling protein (called IRAP or interleukin-1 receptor antagonist protein) into the knee of the rabbit, we can block swelling and we assume, the pain which comes along with that.

The first step is to put the gene for IRAP into your cells. We get your joint cavity lining cells from your joint during the removal of painful swollen joint lining tissue. The joint which this tissue would be removed from would depend on your situation. This procedure would be done for you whether or not you are part of this study. Usually this tissue is sent only to Pathology, and much tissue is wasted. We will use the tissue, which is usually discarded to collect your cells and change them. We will attempt to grow your own cells on glass in our laboratory. We will attempt to change them using a weakened virus as a way to deliver the IRAP gene to the genes of your cells. Many other studies with humans have shown that these weakened viruses can be used as tools to deliver genes to cells in the laboratory. Because the virus has been deliberately disabled, it is normally unable to make more virus, cause disease or affect you or your cells in any way. When your cells are ready (probably 1-2 months after the first procedure), you will come back for an office visit with Dr. Herndon, as normal, to follow your progress after the first procedure. During this visit, Dr. Herndon will give back your modified cells to your knuckle joints through simple injection(s). These injections could be painful enough to require local anesthetic. These are joints which you plan to have replaced with artificial joints in one week. The four knuckle replacement will be undertaken one week after you get your changed cells back. Therefore, all modified cells should be removed from your body. The gene therapy used in this study should not have a lasting effect on you or your disease. However, the joint tissues remove at the time of knuckle joint replacement will be analyzed in the laboratory to see whether the attempt at gene transfer has been successful. You will be followed closely after you undergo your planned four knuckle joint replacement, eventually being seen every two years for the remainder of your lifetime. If you move or Dr. Herndon retires, your records will be forwarded to the physician of your choice. Of course, a referral will be given if asked for.

RISKS AND BENEFITS

1. There is a very small risk that the gene placed in your cells will interfere with an important gene or activate a bad gene. This has never occurred in other studies in

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Patient Initials

humans, although our experience is limited because this form of experimentation is new. If this were to occur it could cause a serious illness, possibly even cancer. To insure your safety, we have made the changed cells sensitive to an antibiotic called ganciclovir. This should destroy the genetically altered cells that were injected into your knuckle joints.

2. You will undergo additional procedures as being part of this study. The collection of joint fluid and injection of the cells prior to the four knuckle replacement may be painful. In addition, you will have 2 tablespoons of additional blood collected at several follow-up visits. There is a small chance that the injected joint could get infected. Such an infection would be treatable by antibiotics. Frequent visits and an experienced surgeon will keep this risk to a minimum.

Thus in the unlikely event that your changed cells we give back to you cause some bad effect, you may be treated with the antibiotic ganciclovir through the vein. This would require hospital admission. Ganciclovir itself has a small associated risk including damage to the kidney. Consultation of an Infectious Disease Specialist (familiar with the use and side effects of ganciclovir) would keep this risk to a minimum in this unlikely circumstance.

3. Premenopausal women who still have their uterus and ovaries (capable of bearing children) will not be accepted into this study.

4. This study will be of no benefit to you personally. Nevertheless, knowledge may be gained to aid in an earlier cure to rheumatoid arthritis and other diseases of the joint.

5. In the event of death at any time in the future, whether through accident, injury or illness, an autopsy will be requested.

ALTERNATIVE THERAPY

The four knuckle joint replacement you will be receiving at the end of this study is the current state-of-the-art treatment for your advanced arthritis and its associated pain. Should a scientifically proven better treatment arise during the course of this study, it may be incorporated into you care.

NEW INFORMATION

If new information, either good or bad, about this treatment comes to the attention of the investigators during the course of this study which may be related to your willingness to participate, it will be provided to you or your representative.

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Patient Initials

COSTS AND PAYMENTS

You will not be charged nor will you be paid for your participation in this study, although you will be charged for the (medical and hospital care) you require independent of this study just as though you were not part of this study. You will not be charged for any research related injury, including ganciclovir treatment if it is required.

COMPENSATION FOR ILLNESS OR INJURY

You will not be compensated for any injury or illness resulting from this study, but any emergency medical treatment which may be necessary will be provided.

CONFIDENTIALITY

Any information about you will be kept in locked files with limited access and not distributed without your permission. Any information about you or your treatment will be treated in the same confidential manner as other hospital medical records. The hospital, the FDA and the National Institute of Health may request to see your records. You consent to publication of any information for scientific purposes so long as your identity will not be revealed.

RIGHT TO WITHDRAW

You may refuse to participate in this study or withdraw any time and that your decision will not adversely affect your care at this hospital/institution or cause a loss of benefits to which you might otherwise be entitled. You may be withdrawn from the study by the investigators if your cells fail to grow adequately in the laboratory, if they fail to make enough of the necessary protein or if they become infected or show other evidence of being unsuitable for use.

****

VOLUNTARY CONSENT

Dr. Herndon has explained all of this to me and has answered all questions I have. I also understand that any future questions I have about this research will be answered by Dr. Herndon who I may call at (412) 687-3900. Any questions I have about my rights as a research subject will be answered by the Office of the Senior Vice President for Health Sciences at the University of Pittsburgh. By signing this form, I agree to participate in this study.

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Patient Initials

SIGNATURE (SUBJECT CONSENT) DATE

SIGNATURE (WITNESS) DATE

Investigator's Certification

I declare that I have personally explained the above information to the patients or the patient's legal representative.

Investigator's Signature

Witness Date

Patient Initials

FF Principal Investigator/Program Director *(Last, first, middle)*:

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Evans, Christopher H.	Professor, Orthopaedic Surgery Molecular Genetics & Biochemistry

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Wales, Swansea, UK	B.Sc.	1971	Genetics/Microbiology
University of Wales, Swansea, UK	Ph.D. 1974		Biochemistry
Free University of Brussels, Belgium	Post-Doc	1974-1977	Molecular Biology
University of Pittsburgh, Pgh., PA	M.A.	1983	Hist/Phil Science

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, ar honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the projec Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership c any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during th past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Postdoctoral Research Fellow, Dept. Molecular Biology 1974-1977
Free University of Brussels, Belgium
Lecturer, Orthopaedic Surgery, Univ. of Pittsburgh 1978-1979
Assistant Professor, Orthopaedic Surgery, Univ. of Pittsburgh 1979-1984
Assistant Professor, Biochemistry (secondary appointment) 1982-1984
Associate Professor, Orthopaedic Surgery/Molecular Genetics & Biochemistry 1984-Present
University of Pittsburgh
Tenure Conferred, University of Pittsburgh 1986
Professor, Departments Orthopaedic Surgery; Molecular Genetics & Biochemistry 1993
University of Pittsburgh

HONORS: Kappa Delta Award (1985); Cabaud Award (1988); Research Prize, German Pain Society (1989); Fellow, Royal Society of Chemistry (1989); Fogarty Senior International Fellow (N.I.H.), 1990.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
PAUL D. ROBBINS	ASSISTANT PROFESSOR

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Haverford College, Haverford, PA	BA	1980	Biology
University of California, Berkeley, CA	Ph.D.	1985	Mol. Biol.
Whitehead Institute, MIT	Post-doc	1990	Mol. Biol.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1986-1990 Post-doctoral Fellow, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts (Laboratory of Dr. Richard Mulligan)
1990-present Assistant Professor, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine
1991-Present Director, Viral Vector Core Facility

Honors and Awards
Magill-Rhodes Scholarship, Haverford College, 1976-1980
High Honors in Biology, Haverford College, 1980
Jane Coffin Childs Memorial Fund for Medical Research Fellowship, 1986-1989

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HUMAN GENE TRANSFER PROTOCOL

9406-075 Freedman, Ralph; MD Anderson Cancer Center, Houston, Texas; *Use of a Retroviral Vector to Study the Trafficking Patterns of Purified Ovarian TIL Populations Used in Intraperitoneal Adoptive Immunotherapy of Ovarian Cancer Patients: A Pilot Study.*

Date of RAC Approval: 6-9-94
Date of NIH Approval: 7-12-94

Our long term **goal** is to develop an effective intraperitoneal adoptive immunotherapy strategy for ovarian carcinoma utilizing TIL-(tumor infiltrating lymphocytes) derived T cells expanded in low concentrations of rIL-2. To achieve this goal it is important to determine whether ovarian TIL concentrate at metastatic tumor sites in vivo. Gene transfer technology offers a sensitive and reliable method to track TIL to tumor sites over a prolonged period of time. The specific **objectives** are to determine: (1) The feasibility of detecting $CD3^+CD8^+$ TIL-derived T cells that have been marked with the gene for Neomycin phosphotransferase encoded in a safety modified retroviral vector (G1Na) at tumor sites after IP injection, and (2) The fold of enrichment of $CD3^+CD8^+$ marked TIL at tumor sites.

Background and Rationale

Epithelial ovarian cancer primarily involves the peritoneal and serosal surfaces, a fact that has made this tumor a target for intraperitoneal (IP) biologic therapies including adoptive cellular therapy (1,2). Preclinical studies from our laboratory suggest that ovarian TIL may represent an active immune response of the host directed against the tumor (3,4). T-cell lines from ovarian TIL expanded in low dose rIL-2 exhibited cytotoxicity primarily against autologous ovarian tumor cells. Our studies have shown that the TCR CD3 complex and CD8 antigens on rIL-2 expanded TIL, and MHC Class I surface antigens on ovarian carcinoma cells are involved in this in vitro cytotoxic activity (3,4). Ovarian TIL-derived T-cell lines have also been shown to produce substantial quantities of cytokines including interferon-γ, TNF-α, and GMCSF following culture in low concentrations of rIL-2 (20 IU) (5). Production of these cytokines by $CD8^+$ T-cell lines can be significantly enhanced by stimulation with autologous tumor cells. TIL may have therapeutic effect in vivo either by direct cell to cell contact or through the production of the cytokines described above. We have completed a pilot clinical protocol in which 8 patients with advanced platinum refractory ovarian carcinoma received IP rIL-2 expanded TIL and low dose rIL-2. Four of these patients had clinical activity after receiving IP TIL plus low dose rIL-2. In a preliminary study utilizing ^{111}In-labeled rIL-2 expanded TIL derived T cells, we observed an increase in the uptake of radioactivity in liver metastases of a patient with ovarian cancer. Neo^R marked TIL expanded in rIL-2 can be detected in both man and in animals for a considerable time after injection (6,10). Previous studies in patients with melanoma have shown that TIL from these patients were successfully transduced with the neo^R gene using the LNL6 retroviral vector (5) which has the same backbone as G1Na, the vector proposed for our marker protocol. The neo^R transduced gene cells could be identified in biopsy material from melanoma metastases even at 64 days after injection. The **hypothesis** to be tested in this protocol is that ovarian rIL-2 expanded purified $CD8^+$ TIL-derived T cells concentrate in metastatic tumor sites in vivo after IV injection.

The experimental approach is briefly described as follows: Marking of the $CD8^+$ TIL-derived T cells by infection with G1Na will be performed after positive selection has been accomplished, and after the purified $CD8^+$ T cells are observed to be multiplying in a T-flask. At this point consenting patients will be registered. A mixture of bulk expanded transduced and nontransduced T cells will be injected IP with rIL-2. Following treatment with the adoptively transferred TIL, (containing transduced and nontransduced cells) and IP rIL-2, biopsies will be obtained at laparoscopy performed at 1 month after injection of the TIL, from previously documented tumor sites and from normal tissues (peritoneum, omentum (if present) and lymph node tissue). Although animal and human studies show that injected TIL can be recovered beyond 2 months, there is evidence that selection of an earlier time point will maximize neo^R detection, with the requirement of a smaller number of patients. DNA will be extracted by a proteinase K phenol procedure and the neo^R specific DNA quantitated by PCR according to Morgan et al (12). A successful result is defined when the mean number of marked cells in tumor tissue is significantly larger than the mean number of marked cells in blood or normal tissues. The study will be completed with a maximum of 9 and a minimum of 5 evaluable patients to determine the fold increase at metastatic tumor sites. The proportions of $CD3^+CD8^+$ T cells will be determined in biopsies utilizing OKT8 and OKT3 moAbs by histochemical procedures that we have established. In addition to these objectives, we will also determine the persistence of neo^R marked T cells in the abdominal cavity during the course of treatment with TIL + rIL-2.

Cancer of the ovary is responsible for more than 12,000 deaths annually in the United States. The disease is usually only diagnosed at a stage when the tumor has spread to involve structures in the abdominal cavity. The standard treatment for ovarian cancer involves surgical removal of the larger tumor masses combined with a combination of chemotherapy drugs. Although new chemotherapy drugs such as taxol are effective, complete responses are few, and toxicity is high. It therefore remains necessary to develop new treatment strategies to improve the survival and quality of life of patients who are afflicted with ovarian cancer.

The results of studies conducted in our laboratories and others have shown that a particular cell type called the tumor infiltrating lymphocyte (TIL) can be isolated from the cancerous tumors that involve the lining of the abdominal cavity and the surface of the intestines. When these cells are removed from the body and are grown together with the patients tumor cells in tissue culture, along with a drug called recombinant interleukin-2 (rIL-2), the TILs will frequently increase to larger numbers and develop an increased capacity to kill tumor cells obtained from the patient. rIL-2 is a protein substance that is almost identical to a natural substance that is found in the body and is produced in very small amounts by the cells of the body's immune system called lymphocytes. We are currently growing large numbers of TILs for the experimental treatment of patients with advanced ovarian cancer in a clinical trial. This type of treatment has been called adoptive cellular immunotherapy. In this trial we purify a particular type of TIL called "$CD8^+$ TIL" which appear to be responsible for the killing of tumor cells in tissue culture in certain cases.

This gene marker protocol will address an important scientific question: whether ovarian TIL-derived T cells that are expanded ex vivo in rIL-2 for intraperitoneal injection, concentrate within metastatic tumors of the abdominal cavity. This information will be helpful in the development of improved therapies for ovarian cancer. In the past this type of question could only be addressed by placing a radioactive label onto the cells and trying to trace the cells using a gamma camera held over the patient. These techniques remain in use but are not sensitive or specific enough to address the questions that are being posed.

Recently, use has been made of defective mouse viruses that have been genetically altered in the laboratory so that they are rendered incapable of multiplying in the patient. These viruses have been used to transfer new genetic information into cells. The gene transfer approach is being used to introduce a stable marker into cells that are being returned to the body for marker studies or in the development of new therapy approaches. Appropriate concerns have been expressed regarding utilization of these defective viruses in patients. Marked improvements in design and in quality control are contributing nevertheless, to safer and more efficient clinical applications.

In our study we intend to use the defective virus G1Na, to transfer the gene for marker DNA. This marker gene is not present in human or mammalian cells. The gene encodes for an enzyme called neomycin phosphate transferase (NPT). The defective virus and related forms have been used on a number of occasions to treat patients without reports of toxicity.

Patients who are enrolled in a study to be treated with TIL will be eligible for the marking protocol. During the growth of TIL in culture a bacterial "marker" gene will be inserted into a portion of cultured TIL cells using a defective mouse retrovirus so that they can be traced after reinjection into the patient. Following injections of a mixture of gene marked and unmarked cells, samples of blood (about 10 cc each) and peritoneal fluid (approximately 100 cc) will be obtained on days 4, 11, 18 and at one month after receiving the TIL injection. One month after receiving the TIL, patient's will undergo a laparoscopy to evaluate their responses to the treatment. At this time biopsies will be obtained from tissues suspected of harboring cancer. Biopsies will also be obtained from certain normal tissues, e.g., peritoneum (lining of abdominal cavity) and lymph node (if easily accessible). These biopsies will be obtained to determine if the TIL have migrated into the tumor. This information might be useful for the development of future treatment strategies in other patients with ovarian cancer. The laparoscopic surgery will be performed to evaluate the response to the treatment and not for the marker study alone. Nine patients may receive the retrovirus modified cells.

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER
DEPARTMENT OF GYNECOLOGIC ONCOLOGY

Protocol: Use of a retroviral vector to study the trafficking patterns of purified ovarian tumor infiltrating lymphocytes (TIL) used in intraperitoneal adoptive immunotherapy of ovarian cancer patients. A pilot study

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PROTOCOL CHAIRMAN:

11-8-93
Ralph S. Freedman, M.D., Ph.D. Date
Department of Gynecologic Oncology

CO-CHAIRMEN:

11/10/93
Chris D. Platsoucas, Ph.D.* Date
Department of Microbiology & Immunology

11/11(93
Albert B. Deisseroth Date
Department of Hematology

The Division of Surgery, Department of Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030

*Room 525●-MS, Temple University School of Medicine, 3400 N. Broad St., Philadelphia, PA 19140

Collaborators:

Edward N. Atkinson, Ph.D. Date
Department. of Biomathematics

Lawrence A. Trissel, B.S. Date
Pharmacy

Cesar H. Carrasco, M.D. Date
Department. of Diagnostic Imaging

Creighton L. Edwards, M.D. Date
Department of Gyn/Oncology

Leela P. Kasi, Ph.D. Date
Department of Nuclear Medicine

J. Taylor Wharton, M.D. Date
Department of Gyn/Oncology

E. Edmund Kim, M.D. Date
Department of Nuclear Medicine

John Kavanagh, M.D. Date
Department of Medical Oncology

Donald A. Podoloff, M.D. Date
Department of Nuclear Medicine

Andrzej Kudelka, M.D. Date
Department of Medical Oncology

Barbara Tomasovic, B.S. Date
Department of Gyn/Oncology

Ruth L. Katz, M.D. Date
Department of Pathology

Micheal A. Nash, Ph.D. Date
Department of Gyn/Oncology

The Division of Surgery, Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title) (Two lines not to exceed 75 characters per line)

Use of a retroviral vector to study the trafficking patterns of purified ovarian TIL populations used in intraperitoneal adoptive immunotherpy of ovarian cancer patients - a pilot study

Study Chairman: RALPH S. FREEDMAN, M.D., Ph.D.

Patient Eligibility: (Twenty lines not to exceed 75 characters per line)

(1) **Patients who participate in this protocol must also be registered on the treatment protocol ID92- 015 (T92-0093).**
(2) Patients with histologically documented ovarian carcinoma or peritoneal carcinomatosis who have persistent or recurrent disease after at least one platinum based chemotherapy and less than 2 cm residual.
(3) Accessible tissue samples for TIL preparation.
(4) Easily measurable disease preferred but not essential
(5) Age 16 years +.
(6) ECOG PS 0-1 Karnofsky 100-70%.
(7) Adequate organ functions, (a) AGC $\geq$ 1500/cu mm; platelets > 100,000 cu/mm; (b) Bilirubin < 1.5 mg/dl, creatinine $\leq$ 1.5 mg/dl, or creatinine clearance > 60 ml/min.
(8) No significant heart disease.
(9) Adequate pulmonary reserve.
(10) No active infection.
(11) No contraindication to pressors.
(12) At least 4 weeks after chemo hormones or RT.
(13) Negative HIV and HB_S Ags.
(14) Signed informed consent.

Exclusions:
(1) Intestinal dysfunction or uncorrectable obstruction.
(2) Prior abdominal irradiation (pelvic or small treatment field may not exclude).
(3) Extreme adhesions preventing free flow of fluid.
(4) Prior therapy with I.P. IL-2.
(5) Prior I.P. chemotherapy unless free of extensive adhesions.
(6) Contraindication to surgical exploration.
(7)) Patients with organ allografts and those requiring corticosteroids for intercurrent disease.
(8) Other malignancy <10 years in remission, other than basal or squamous cell cancer of the skin.
(9) Significant medical/psychiatric disorders.

Treatment Plan: (Include dose adjustment) (Twenty lines not to exceed 75 characters per line)

All patients will be registered at the Department of Gynecology Protocol Office (713)- 792-6828. TIL will be obtained from tumor biopsies or abdominal cavity irrigations obtained at tumor reductive surgery or laparoscopy performed for the evaluation of response to prior chemotherapy or for surgical tumor reduction. TIL-derived T cells will be cultured in rIL-2 supplemented medium. During the culture phase, $CD8^+$ TIL will be purified by positive selection and an aliquot of these cells infected with a retroviral vector containing the gene for neomycin resistance (G1Na). Purified rIL-2 expanded $CD3^+CD8^+$ TIL (mixture of transduced and nontransduced cells) will be administered I.P. over 30 - 60 minutes with 0.033 mg ($6x10^5$ IU) IL-2 Cetus in 300 - 500 ml D5 1/4 normal saline plus 0.1% human albumin USP. rIL-2 alone at a dose of 0.033 mg/m^2 will be given I.P. as a bolus on days 2-4, 8-11, and 15-18. Laparoscopy will be performed in patients who have no clinical or radiologic evidence of disease 1 month after receiving the IP injected cells.

Patient Evaluation: (Twenty lines not to exceed 75 characters per line)

Physical examination, history, performance status, tumor status assessment, complete blood count, differential, platelets, chemistry survey (total bilirubin, SGOT, LDH, alkaline phosphatase, creatinine, BUN) Ca 125, vital signs, weight, PT and PTT, FEV_1, ABGs, EKG, CXR, cardiac stress test, UA and culture, HB_S Ag. HIV, lymphocyte subpopulations, abdominal ascites for cytology, and DNA flow analysis. (These studies are required for the treatment protocol).

Statistical Considerations: (Twelve lines not to exceed 75 characters per line)

A justification is provided for undertaking a pilot study of 9 patients. PCR quantification in tumor and normal control sites will be performed according to the analytic methods of Morgan et al. A successful outcome in this experiment is defined when the mean number of marked cells in tumor tissue is significantly larger than the mean number of marked cells in either the blood and the normal tissue. The mean referred to here is the average of the measurements taken in triplicate. If the first five patients exhibit a successful response, then there is significant evidence at the .05 level to reject the null hypothesis that the response rate is less than or equal to 1/2. In order to collect evidence that the probability of success is less than .30, 9 evaluable patients are needed. If all 9 patients have an unsuccessful outcome; i.e., the mean number of marked cells in tumor tissue is less than or equal to the mean number of marked cells in both the blood and the normal tissue, then there is evidence to reject the null hypothesis that the probability of success is greater than or equal to .30.

Objectives: (Twelve lines not to exceed 75 characters per line)

1. The feasibility of detecting $CD3^+CD8^+$ TIL-derived T cells that have been marked with the gene for neomycin phosphotransferase encoded in a safety modified retroviral vector (G1Na) at tumor sites after IP injection, and

2. The fold of enrichment of $CD3^+CD8^+$ marked TIL at tumor sites.

1.0 OBJECTIVES

Our long term goal is to develop an effective adoptive immunotherapy strategy for epithelial ovarian cancer (EOC) utilizing T-cell lines derived from ovarian tumor infiltrating lymphocytes (TIL). To achieve this goal it is important to determine that these ovarian TIL lines concentrate at metastatic sites *in vivo*. Gene transfer technology now offers a reliable and sensitive method to track TIL to tumor sites over a prolonged period of time. We therefore plan to determine:

1.1 The feasibility of detecting $CD3^+CD8^+$ TIL-derived T cells that have been marked with the gene for Neomycin phosphotransferase encoded in a safety modified retroviral vector (G1Na) at tumor sites after IP injection, and

1.2 The fold of enrichment of $CD3^+CD8^+$ marked TIL at tumor sites.

2.0 BACKGROUND AND RATIONALE

2.1 Ovarian Cancer

EOC is responsible for the highest mortality among patients with gynecologic malignancies with a 39% overall survival at 5 years (1). Moreover only 20% of patients with EOC diagnosed in stages III and IV survive 5 years. Postsurgery combination chemotherapy regimens that includes cisplatin or carboplatin produces an overall initial response rate of 60-80% (2,3). The majority of these patients ultimately progress or relapse due to primary or acquired drug resistance (4). There is evidence that salvage therapy with intraperitoneal IP approaches such as high dose cisplatin may benefit certain patients with minimal residual disease (5). In addition, recent phase II data on the activity of systemically administered taxol in platinum refractory ovarian cancer patients looks promising, although there are only few complete responses and considerable toxicity with this treatment. In consideration of the above modest survival rates achieved, current treatment modalities for ovarian cancer leave much to be desired.

2.2 Cytokine activated killer cells

Potentiation of an autologous tumor-specific immune response is a central goal of the biological therapy of cancer. In a variety of murine models therapy with the cytokine interleukin-2 (rIL-2) alone can result in significant tumor regressions (reviewed in 6). Potential mechanisms involved in this antitumor effect include the activation of lymphokine activated killer (LAK) cells, generation of cytotoxic T-lymphocytes against the tumor, and the secondary induction of other cytokines such as tumor necrosis factor α (TNF) or rIFN-γ. Clinical responses, including durable complete remissions were observed in cancer patients treated with ex-vivo activated LAK cells and high doses of rIL-2. Complete plus partial response rates in initial clinical trials approximated 20 percent in melanoma, 30 percent in renal cell carcinoma, 10 percent in colorectal carcinoma (7,8), and 20 percent in ovarian cancer (9,10). However, these responses are comparable to those observed by rIL-2 alone. Individual responses have been noted in patients with other malignancies including lung carcinoma, Hodgkin's and non-Hodgkin's lymphomas, but comparatively few patients have been treated with malignancies other than melanomas, renal cell, or colon carcinoma. While an advantage of LAK cells is the relative ease with which they may be generated by cultivation of peripheral blood lymphocytes obtained by lymphocytophoresis in culture medium containing high doses of rIL-2, a significant disadvantage is the fact that these cells do not target to sites of malignant disease in radiolabeled trafficking studies, a fact that underlines the lack of specificity by LAK cells. This failure to concentrate at tumor sites may be partially responsible for the results noted to date in comparative trials of high dose rIL-2 with or without ex-vivo activated LAK cells in melanoma, which demonstrate little or no advantage to the added LAK cells (7). Although LAK cells alone do not appear to add substantially to rIL-2 treatment alone (11), the treatment with TIL + rIL-2 have been

associated with objective clinical responses in patients with melanoma (12) and ovarian cancer (13).

2.3 Ovarian tumor specific TIL studies conducted at M.D.A.C.C.
We have developed T-cell lines from ascites (14,15) or from solid tumors (16,17) of EOC in low concentrations of rIL-2. These T-cell lines were either $CD3^+CD8^+CD4^-TCR\alpha\beta^+$ or $CD3^+CD4^+CD8^-TCR\alpha\beta^+$. Certain T-cell lines of the $CD3^+CD8^+CD4^-$ phenotype exhibited cytotoxicity primarily against autologous ovarian tumor cells (14-16). Furthermore, we have shown that the T-cell receptor CD3 complex and MHC Class I antigens on tumor cells are involved in this tumor specific activity (15). $CD3^+CD8^-CD4^+$, rIL-2 dependent T-cell lines exhibited MHC unrestricted cytotoxicity (15-17). Other investigators have also developed T-cell lines from ascites or from solid tumors of patients with EOC (18-22). A number of these T-cell lines were developed in higher concentrations of rIL-2 and expressed only nonspecific cytotoxicity (18-21). Other T-cell lines developed from 5 of 6 ovarian cancer patients by Peoples et al. (22) in low concentrations of rIL-2 exhibited autologous tumor specific activity. This is in agreement with our previous reports (14-16).

2.4 Generation of large numbers ($1x10^{10}$ to $1x10^{11}$) of TIL- derived T cells for adoptive immunotherapy
Large numbers of rIL-2 expanded TILs have been produced utilizing automated closed systems (23,24). We have produced large numbers ($1x10^{10}$ to $1x10^{11}$) of TIL-derived T-cells from patients with ovarian carcinoma using a four-step expansion procedure (17). This procedure employs 24 well culture plates, T-flasks, polyolefin gas permeable bags (PGPB) (23) and a hollow fiber bioreactor (artificial capillary culture system) (ACCS) (17). Mixtures of TIL and autologous tumor cells were prepared either from malignant ascites by centrifugation on Ficoll/Hypaque or from solid tumor specimens by enzymatic dissociation. The mixtures were cultured in AIM V medium supplemented with 600 IU/ml rIL-2 (CETUS) in 24-well flat-bottomed tissue culture plates. After one week in culture the expanded TIL were transferred to T-75 Falcon plastic tissue culture flasks and after two to three weeks approximately 2.5 to $5.0x10^8$ TIL were transferred into gas permeable polyolefin bags (Baxter). After $1x10^9$ to $2x10^9$ cells were generated (within a few days) the TIL were transferred with the media into a BRB-23B1 cartridge of the ACCS (17) (Cellco, Germantown, MD). The TIL were harvested at a mean of 47 days from inception of the culture with a mean fold of expansion of 8,044-fold (17). TIL expanded in the ACCS may be either $CD3^+CD4^+TCR\alpha\beta^+$ or $CD3^+CD8^+TCR\alpha\beta^+$. It has been shown (data submitted to Master File by Cellco, Germantown, MD) that a modified cellulose fiber with a 50% molecular weight cut-off of 30 Kd will support the expansion of T-cells ($CD8^+$ and $CD4^+$). We will utilize the AM-FP13E0 cartridge because it requires substantially lower concentrations of rIL-2 than that required by the BRB-23B1.

2.5 A clinical trial in patients with ovarian carcinoma utilizing rIL-2 expanded TIL and low concentrations of rIL-2
A pilot study was conducted in patients with advanced epithelial ovarian carcinoma, who were refractory to platinum based chemotherapy to determine the feasibility and clinical effects of a schedule of intraperitoneal (IP) tumor infiltrating lymphocytes (TIL) expanded in recombinant interleukin-2 (rIL-2), and low dose rIL-2 IP. TIL were expanded from solid metastases or malignant effusions in serum free AIM V medium supplemented with low concentrations (600 IU/ml) of rIL-2 using the four-step expansion method described above. Patients received IP TIL suspended in 5% dextrose in 0.2% sodium chloride containing 0.1% human albumin and $6x10^5$ IL rIL-2 on day 1, followed by $6x10^5$ IU rIL-2/m^2 body surface area, administered daily by bolus injection, on days 2 to 4, 8 to 11, and 15 to 18. In the absence of disease progression two additional four day cycles of IP rIL-2 were administered. Patients (n = 3) whose TIL failed to grow *in vitro* received IP IL-2 alone. Eight patients received rIL-2 expanded TIL (10^{10}-10^{11} range) plus rIL-2 followed by several cycles of rIL-2 alone (see above). One of these patients was treated twice with TIL plus rIL-2.

Expanded TIL were primarily $CD3^+CD4^+TCR\alpha\beta^+$ (8 TIL-derived T-cell lines) and primarily $CD3^+CD8^+TCR\alpha\beta^+$ in one TIL-derived T-cell line. Eleven patients (8 treated with TIL plus rIL-2 and 3 patients treated with rIL-2 alone) received a total of 38 cycles of rIL-2 without TIL. Grade 3 clinical toxicity (peritonitis) occurred in 1 of 9 four day TIL plus rIL-2 cycles and 1 of 38 four day cycles of rIL-2 alone. Grade 3 anemia occurred in 1 of 9 TIL plus rIL-2 cycles and 3 of 38 cycles of rIL-2 alone. Two patients required blood transfusions. There were no measurable responses, however, 4 of 8 patients treated with IP TIL plus rIL-2 had some indication of clinical activity: ascites regression (2 patients), tumor and CA-125 reduction (1 patient), and surgically confirmed stable tumor and CA-125 values (1 patient). The schedule of IP TIL plus low dose rIL-2 shows manageable toxicity and is worthy of further evaluation in patients with EOC who have less tumor burden.
The most significant toxicities that have been described with IP adoptive cellular therapies are catheter related infections (25-28) and peritoneal fibrosis (25,27,28). These problems have been overcome by attention to the following details: (a) the use of lower doses and duration of IP rIL-2 such as we currently employ, i.e., $6x10^5$ IU/m^2/4 day cycle and, (b) restricting IP treatments to 3 cycles/course; and removal of IP catheters after 18 days; and (c) instituting prophylactic antibiotics at the time of IP catheter insertion. Using this approach we have observed no catheter related infections by performing microbiologic cultures on the tips of catheters removed from a number of patients after 18 days.

2.6 Purification and expansion in rIL-2 of $CD8^+$ or $CD4^+$ TIL-derived T-cell lines
Expansion of ovarian TIL in rIL-2 may result in T-cell lines that are primarily $CD3^+CD8^+CD4^-\alpha\beta TCR^+$ or $CD3^+CD4^+CD8^-\alpha\beta TCR$ or mixtures of $CD3^+CD8^+$ and $CD3^+CD4^+$ T-cell lines. Purified $CD4^+$ and $CD8^+$ T-cell lines have been developed from rIL-2 expanded TIL using appropriate antibody coated flasks (CELLector devices, Applied Immune Sciences (AIS) Menlo Park, CA) (29). When rIL-2 expanded TIL reached $2x10^8$ cells they are transferred in complete AIM V medium (includes rIL-2 at 600 IU/ml) to a CELLector-TIL sterile polystyrene chamber with anti-CD8 mAb covalently bound to the inner surface. $CD8^+$ cells are separated from the mixture of $CD4^+$ and $CD8^+$ rIL-2-expanded TIL by positive selection. This device is further described in reference (29) and in a master drug file with the FDA. Within 2-4 days the cells detach from the matrix in the chamber and continue their expansion as described in our procedure for unseparated rIL-2 expanded T-cell lines (17).

2.7 IP rIL-2 administration in combination with TIL.
Ovarian TIL-derived T-cell lines require the presence *in vitro* of low concentrations of rIL-2 to sustain proliferation and for other functions including cytotoxicity (14,15,18) and in the production of cytokines (30). Since activated TIL derived T cells may require several days to concentrate at tumor sites (31) it is probably necessary to administer these cells with exogenous rIL-2 in order to maintain a suitable micro-environment for the cells after injection. Pharmacokinetic studies have shown that rIL-2 can be detected for a prolonged time after IP injection with an estimated half-life of 22 hrs following IP bolus injection (32). This compares with a half-life of only minutes when rIL-2 is administered by IV bolus injection.

2.8 The use of neo^R marked cells to study trafficking of $CD8^+$ TIL-derived T cells in ovarian carcinoma patients who are receiving IP adoptive immunotherapy.
We will determine whether purified $CD3^+CD4^-CD8^+TCR\alpha\beta^+$ ovarian TIL-derived T cells marked with neo^R that are expanded in rIL-2 (600 IU/ml) for the companion treatment protocol ID92-015 (T92-0093) concentrate in metastatic tumor sites in the peritoneal cavity of patients with epithelial ovarian carcinoma.

2.8.1 The treatment protocol.
The purified $CD8^+$ T cells will be expanded to large numbers (10^{10} to 10^{11} range) in low concentrations of rIL-2 (600 IU/ml) by a four-step method that we have developed (18). Ovarian cancer patients who have failed at least first-line chemotherapy will be eligible to receive IP injections of rIL-2 expanded T cells together with low doses of rIL-2 under the companion treatment protocol ID92-015 (T92-0093). This protocol is approved by the Cancer Therapy Evaluation Program at the National Cancer Institute and by the M. D. Anderson Surveillance Committee (IRB). Under this protocol, patients will undergo as part of their clinical evaluation: (1) serial sampling of cells from the peritoneal catheter used for administering TIL + rIL-2 into the peritoneal cavity, and (2) laparoscopic evaluation for clinical response one month after IP treatment with $CD8^+$ T cells.
Metastatic lesions of ovarian cancer are usually observed as surface lesions on the peritoneum of the abdomino-pelvic cavity and on the serosa of the intestines. These surface metastases shed tumor cells and TIL into the abdominal cavity which are easily retrieved for growing TIL-derived T-cell lines as we have shown in previous experiments (14-17). The situation of these metastases are monitored by video laparoscopy. The metastatic lesions of ovarian cancer are in direct contact with TIL-derived T cells utilized in IP adoptive immunotherapy. Only the patients who are enrolled on the above treatment protocol will be eligible to have their rIL-2 expanded TIL marked with the neo^R gene as described in this protocol.

2.8.2 Rationale for using neo^R transduced cells *in vivo*.
$CD8^+$ TIL derived T cells may exhibit a therapeutic effect in vivo by a number of different mechanisms. These TIL may have therapeutic effects either directly by cell to cell contact or indirectly through the production of a number of cytokines such as interferon-γTNFα or GMCSF. Our studies have shown that $CD8^+$ ovarian T cells may exhibit HLA class I restricted autologous tumor specificity (14,15) or may produce cytokines in a class I restricted manner in response to autologous tumor cells (30). These cytokines may exhibit antitumor activity by one or more of the following mechanism(s): (a) by direct lysis of ovarian tumor cells; (b) by enhancing HLA Class I and II expression on tumor cells that can now be recognized more easily by MHC restricted T cells; (c) by inducing the differentiation of pre-effector T cells to effector T cells. The objectives of this protocol are to determine whether the *in vitro* expanded purified $CD8^+$ T cells home to the tumor sites. Data from these experiments will enhance our understanding of how these cells work *in vivo* and permit the development of more effective adoptive immunotherapeutic strategies for patients with ovarian cancer.
Previous clinical experiments in melanoma conducted with TIL labeled with ^{111}Indium oxine have provided very limited information on TIL trafficking, largely because of the short half-life of ^{111}Indium and because ^{111}Indium is preferentially taken up by the liver (31). For these reasons we were encouraged by experiments utilizing retroviral vectors for marking of TIL that were conducted by Rosenberg and associates (33-37), and reviewed by Anderson (38) and by Miller (39). These studies have shown that certain safety-modified nonreplicating retroviral vectors which contain the gene for the neomycin phosphotransferase enzyme (neo^R) can be used to mark and to trace cultured TIL-derived T-cells *in vivo* utilizing sensitive PCR detection methods. Neo^R marked TIL can be detected in both man and animals for a considerable time after in vivo administration. Therefore marking TIL with the neo^R gene using retroviral vectors is an excellent way to study the homing and survival of these cells at the tumor site (33-37). Animal studies reported by Culver et al (33), reported that a positive signal for neo^R was detected between 4 and 24 months in 3 of 4 monkeys who received IV injections of cultured T-cells infected with a neo^R containing retrovirus vector. Alexander et al (40) recovered TILs from mice that had been cured by treatment with TILs up to 119 days after they had received injections of these TILs. By administering rIL-2 concurrently to these animals TIL recovery was increased at all time points at the tumor sites. Moreover, when mice whose pulmonary metastases had been cured with TIL, were rechallenged with tumor, the donor TIL were found to concentrate at the tumor donor sites showing that these cells retained memory characteristics. Rosenberg reported a clinical trial involving patients with

malignant melanoma who were treated with rIL-2 expanded neo^R transduced and non transduced TIL. The proportion of neo^R transduced cells ranged from 1 - 11% and the number of transduced cells that were injected raged from 0.6 to $1.8x10^8$ (37). The neo marked TILs were detected in cells from metastatic tumors even at 64 days after IV injection of the TIL.
Certain safety concerns remain regarding the clinical application of retroviral vectors in general, a larger clinical experience with these vectors and the introduction of stricter quality assurance measures utilized in the production of these vectors is providing reassurance about their safety (41). A recent report states that more than 110 patients (from different studies) have received cells infected with safety-modified retrovirus vectors without any adverse reports of clinical toxicity (42).

2.8.3 Experimental data demonstrating our ability to conduct *in vivo* studies with neo^R marked TILs.
We have established in our laboratory all the methods needed to accomplish the objectives of this protocol (see 3.2(d) p. 7-9 and **Appendix 1**). These methods have been repeated many times and have been found to be reproduceable and employable as a routine. Results from our experiments are provided in Appendix 1 and are also summarized briefly below. Purified ovarian $CD8^+$ TIL-derived T-cell lines were infected with G1Na which has the same backbone as the retroviral vectors LNL6 and N2. Infection of $CD3^+CD8^+$ TIL with G1Na were accomplished with 3 infections at a virus concentration of 2-4 MOI per cell. Oligonucleotide primer sequences utilized for the detection of the 424 b.p. PCR product of the neo^R gene were provided by Ed Otto (G.T.I., Gaithersburg, MD). The neo^R gene segment was detected in the G1Na transduced cells using a sensitive PCR assay based on procedures established by Morgan et al (43). The 424 b.p. gene product was detected by ethidium bromide (EtBr) staining and by Southern blotting followed by detection with ^{32}P labeled neo^R probe. Some additional refinements to the PCR method have been incorporated following suggestions by Ed Otto (G.T.I.). Assays performed in our laboratory are capable now of detecting a single copy of neo^R, i.e., the number of neo^R gene copies expected from an analysis of a 2 μg DNA sample obtained by diluting neo^R transduced cells in nontransduced cells at a ratio of 1:100,000 cells (43). The following modifications have led to a significant improvement in our retrovirus vector infection protocol: (a) infections with G1Na were performed at an MOI of 3, (b) three infections 24 hrs apart were performed after a vector incubation period of 6 hrs. (c) The culture medium was replaced with complete and conditioned AIM V medium between infections. In studies previously reported by Rosenberg and associates (37), infections with LNL6 were performed at an MOI of 2 and only two infections were utilized. Under the experimental conditions that we have used, the numbers of $CD8^+$ T cells transduced with neo^R were at least 10% in 4 of 5 $CD8^+$ TIL-derived T-cell lines and 5% in one line **(Appendix 1)**. Moreover, no substantial differences were found in the lymphocyte phenotypes or in the rIL-2 dependency of these T-cell lines following infection with G1Na in comparison to those that were not infected. *In vitro* cytotoxicity was also not diminished after infection. Because of the improved transduction efficiency, the number of neo^R marked T cells injected IP will be approximately $5x10^8$ cells or higher.

2.8.4 Summary of marker protocol
Marking of the $CD8^+$ TIL-derived T cells by infection with G1Na will be performed after positive selection has been accomplished and the purified $CD8^+$ T cells are observed to be multiplying in a T-flask. At this point consenting patients will be registered. A **separate informed consent** (attached to this protocol) will be obtained. The bulk expanded transduced and nontransduced T cells will be injected IP with low dose rIL-2. Following treatment with the adoptively transferred TIL, and IP rIL-2, biopsies will be obtained at laparoscopy performed at 1 month after injection of the TIL, from previously documented tumor sites and from normal tissues (peritoneum, omentum (if present) and lymph node tissue). Although animal and human studies show that injected TIL can be recovered beyond 2 months we believe that selection of an earlier time point will maximize detection with a

smaller number of patients being required. DNA will be extracted by a proteinase K phenol procedure and the neo^R gene quantitated by PCR to determine the fold increase at metastatic tumor sites. The proportions of $CD3^+CD8^+$ T cells will be determined in biopsies utilizing OKT8 and OKT3 moAbs by histochemical procedures that we have established (44). In addition to these objectives, we will also determine the persistence of neo^R marked T cells in the abdominal cavity during the course of treatment with TIL + rIL-2.

3.0 BACKGROUND DRUG INFORMATION AND PREPARATION OF NEO^R TRANSDUCED CELLS

3.1 Proleukin recombinant interleukin-2 a (human) (Chiron-CETUS)

A clone containing the rIL-2 gene was derived from ds cDNA synthesized from mRNA extracted from the human Jurkat cell line. The rIL-2 protein is produced by Escherichia coli containing the pBR322 plasmid into which the gene was inserted.

Proleukin differs from native rIL-2 in that it is not glycosylated and has no N-terminal alanine, and a serine substituted for cystine at the amino acid position 125. Its molecular weight is approximately 15,600 daltons.

3.1.1 Reconstitution

Proleukin is supplied as a lyophilized cake in 5 cc vials containing 1 mg protein.

Reconstitution Directions: IL-2 (Chiron), NSC #373364, is provided as a lyophilized powder. Each 5 cc vial has a labeled strength of 1.3 mg (22 million IU). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml. Direct the diluent against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. The resulting solution should be a clear, colorless liquid.

Reconstituted Proleukin is suitable for intravenous injection directly or may be diluted as necessary in volumes of 50 mL to 500 mL with 5% Dextrose injection, USP with 0.1% Albumin Human, USP. When diluting, the Albumin Human, USP should be added to the 5% Dextrose injection, USP prior to the addition of the reconstituted Proleukin.

For TIL administration dilution of original reconstituted rIL-2 is performed in 5% dextrose in 0.2% Normal Saline with 0.1% Albumin Human, USP.

Reconstituted and diluted Proleukin contain no bacteriostatic agents. Sterile Technique should be carefully observed during their preparation and administration.

3.1.2 Stability

Lyophilized Cake in Vial. Stable at 2-8°C for 18 months from date of manufacture. Protect the lyophilized material during extended storage from excessive exposure to light.

Proleukin, diluted for intravenous administration in 5% Dextrose injection, USP, in a plastic bag (e.g. Viaflex, manufactured by Travenol Laboratories, Inc.) containing 0.1% Albumin Human, is stable for 48 hours at refrigerated and room temperature 2° to 30°C (36° to 86° F).

3.1.3 Suppliers

All rIL-2 for this project will be supplied by the National Cancer Institute. CELLector T-150 Cell Selection Procedure device will be supplied by Applied Immune Sciences Inc., Menlo Park, CA.

3.1.4 Incompatibility

Reconstitution or dilution with Bacteriostatic Water for injection, USP or 0.9% Sodium Chloride injection, USP should be avoided because of precipitation (see 5.4 for special consideration for use of rIL-2 with TIL). Proleukin for injection should not be mixed with other drugs.

Studies of bioactivity after dilution of reconstituted Proleukin in diluents of 5% Dextrose injection, USP containing varying percentages of Albumin Human, USP have been performed. A final concentration of 0.1% Albumin Human, USP, in 5% Dextrose injection, USP, has been found to be optimal for full recovery of Proleukin, rIL-2 biological activity. Some loss of bioactivity has been observed with increasing percentages of Albumin Human, USP. Dilution of reconstituted Proleukin rIL-2 with 2% Albumin Human, USP in 5% Dextrose injection produced solution with approximately 90% of the bioactivity measured with 0.1% Albumin in the diluent.

It is recommended that in-line filters not be used for delivery of Proleukin in administration devices. Bioassays have shown significant loss of Proleukin when filters are used.

Note: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

3.1.5 Sterility

Although reconstituted Proleukin is stable, the product does not contain a preservative and accidental microbial contamination may occur. Use of water containing preservatives (benzyl alcohol or parabens) to reconstitute Proleukin results in the formation of a precipitate.
Vials should be entered once only for reconstitution and once only to remove an aliquot for further dilution in order to minimize the chance of contamination. Sterility cannot be guaranteed after the vial has been entered. We recommend prompt administration to patient following reconstitution. Any unused portion should be discarded.

3.2 Collection, Expansion and Administration of TIL

Cell suspensions of TIL and tumor cells are prepared from peritoneal fluids or solid tumor metastases, excised from patients with ovarian cancer as we have described previously (17).

(a) Suspensions of TIL and tumor cells from peritoneal fluid or washings are washed twice and resuspended at $5x10^5$ cells/ml in AIM V serum-free medium (Gibco, Grand Island, NY) with 600 IU/ml rIL-2 in 24 well Costar plates. Cells are expanded to approximately $1x10^9$ in plastic bags (PL732 plastic Fenwal Labs, Deerfield, IL), and cultured at 37°C with 5% CO_2. Approximately every 5 days, new AIM V medium with rIL-2 is added, and the cell density is maintained at $< 2x10^6$ TIL/ml during the entire culture.

(b) Enzyme digestion procedure for solid specimens

Transfer tissue (1 to 4 grams) to 100 mm sterile glass petri dish. Add 10 ml AIM V medium (Gibco). Using sterile scalpels mince the tissue to 1 mm size pieces. Transfer minced tissue along with medium to sterile spinner flask. Add 2.5 ml of 3% collagenase type I (Sigma C-0130) in AIM V medium and 5 ml of 0.02% DNase I, type II (Sigma D-4527) in AIM V medium. Place on stir plate at medium speed in 37°C incubator (CO_2 not necessary) for 16 hours or overnight. Less than 1 gram of tissue may take a shorter time. Transfer contents of spinner flask to a 50 cc centrifuge tube. Spin at 450xg for 5 minutes. Wash once with AIM V. Resuspend pellet in AIM V culture medium and rIL-2 as above in Section 3.2(a).

(c) Production of purified $CD3^+$ $CD8^+$ TIL

The T-150 CELLector devices will accommodate up to $9x10^7$ $CD8^+$ cells of interest (incorporated in Standard Operating Procedure from AIS) (detailed procedure also on file with Regulatory Affairs at NIH, Room 4B11). $CD3^+$ $CD8^+$ TIL are purified from the expanding TIL culture by positive selection when the number of cells in culture reaches approximately $4.5x10^8$. In some cases negative selection may be required to remove double stained cells. An aliquot of $CD8^+$ TIL harvested from the CELLector device are infected with G1Na - (neo^R) vector as described in (d). The remaining cells that are not transduced will be transferred to one of two ACCS cartridges at $1x10^9$.

(d) Preparation of neo^R marking vector and conditions of exposure to TIL cells

(1) The safety-modified marking vector G1Na is a replication incompetent retroviral vector which has been extensively used in marking human hematopoietic cells for infusion into human subjects as part of therapeutic procedures. This vector is prepared by Genetic Therapy, Inc. of Gaithersburg, Maryland as a clinical grade cell-free supernatant from the PA317/G1Na40 producer cell line in DMEM medium at a titer of > $1x10^5$ transducing units/cc. The procedures for preparing this vector have been previously reviewed and approved by the FDA..

The conditions of exposure of the $CD8^+$ (and $CD4^+$) T-cell lines to the marking vector G1Na are as follows:

(2) The purified TIL cells detached from the CELLector flask (in the range of $1x10^7$ to $2x10^8$) will be divided into two parts. One part of approximately $1x10^6$ cells will be used for marking with neo^R , and these cells will be expanded in one ACCS cartridge.. The second part comprised of the remaining cells will not be infected but will be expanded directly in a second ACCS.

(3) After thawing, the vector supernatant will be added in a laminar flow hood dedicated for clinical studies to the one portion of $CD3^+CD8^+$ cells in rIL-2 to reach a final concentration of 2-4 MOI per cell. This will require 2-4 cc of G1Na supernatant per infection, assuming there are $1x10^6$ cells. The viral supernatant is added with rIL-2 (600 IU/ml) to the cells for 6 hours. The cells are pelleted and resuspended in conditioned complete AIM V medium up to 24 hours (1/3 conditioned AIM V and 2/3 new AIM V + rIL-2 (600 IU/ml). The infections are repeated twice at 24 hour intervals. Between infections cells will be maintained in conditioned complete AIM V medium (i.e., containing rIL-2) after removing viral supernatant. rIL-2 concentration of 600 IU/10^6 cells will be maintained in the viral supernatant.

(4) Preservative-free Protamine sulfate (I.V. grade, material), Elkins-Sinn, Inc. NDC# 0641-2554-41, at 10,000 ug/ml for I.V. use) will be added at 4 ug/ml during infection.

(5) Following incubation, the cells will be collected from the supernatant and pelleted by spinning at 450 x g for five minutes, washed three times in complete AIM V and then counted.

(6) The neo^R transduced cells will undergo completed expansion in a parallel ACCS cartridge.

(7) Confirmation of transduction with the neo^R gene will be obtained by the polymerase chain reaction (PCR) using two neo^R gene specific oligonucleotide amplification primers (See Appendix) .

1. 5'GGTGGAGAGGCTATTCGGCTATGA3'
2. 5'ATCCTGATCGACAAGACCGGCTTC3'

(8) Determination of the percentage of neo^R transduced cultured $CD3^+CD8^+$ T cells.
The percentage of $CD8^+$ TIL that express the neo^R gene product will be estimated by the PCR procedure and analysis as we have described (see Appendix 1 for validation data from our laboratory). DNA will be extracted by a standard proteinase K/phenol method. Neo^R transduced K562 cells were obtained from Dr. A. Deisseroth. These K562 neo^R cells were cloned (clone 1), and the clone which expresses one copy of neo^R/cell clone is now established as a laboratory standard which is utilized to estimate the number of transduced $CD8^+$ T cells in the cultured T-cell lines. Detection of the 424 b.p. gene product is performed by ethidium bromide (EtBr) staining. The neoamplification shows the expected product in the neo transduced TIL DNA and is appropriately negative in the blank and NT reactions. The relative intensity of the neo^R bands was standardized by comparison of the actin bands obtained from TIL DNA in K562 DNA.

(e) Tests Performed on Transduced TIL (Certificate of Analysis)
The transduced cells from all patients will undergo the following testing:

Test	Method	Limits
Assay for residual tumor	Cytology	Negative
Viability*	Trypan Blue	≥ 70%
Microbiologic Studies*	Gram stain	No bacteria
	Bacterial culture and sensitivity	Negative†
Virology**	S+/L-assay	Negative†
	3T3 amplification	Negative
DNA analysis	PCR or Southern Blot	Provirus present†
Autonomous growth	IL-2 withdrawal	Negative†
Endotoxin*	Limulus	< 5†
Cytotoxicity*	^{51}Cr release	†
Phenotype*	FACS	†

*Described in detail in Methods and Validation studies submitted to Regulatory Affairs (see ID92-015, T92-0093, October 1, 1992), and June 15, 1993.
**Will be done by GTI by methods used in previous studies.
†Results will be available after treatment of the patient.

4.0 PATIENT ELIGIBILITY

4.1 **Patients who participate in the marker protocol must have been registered under the treatment protocol ID92-015 (T92-0093).**

4.2 Patients with histologically documented ovarian carcinoma who have persistent or recurrent disease after at least one platinum based chemotherapy regimen, and < 2 cm residual. Patients with primary peritoneal carcinoma e.g., (serous or mucinous) are also eligible after failing cisplatin based chemotherapy. (In patients evaluated at

"second look" after first line chemotherapy, tumors may be decreasing, stable or increasing). Prior taxol exposure will not exclude patients.

4.3 Tissue samples for preparing TIL will be obtained from two tumor biopsies or irrigation of the abdominal cavity at surgical exploration or laparoscopy as described in protocol ID92-015 (T92-0093). **Patients will become eligible for the marker protocol once cultured CD8$^+$ T cells are obtained**.

4.4 At least 16 years of age.

4.5 Ambulatory with good performance status (ECOG PS 0-1; Karnofsky PS 100-70%).

4.6 Adequate organ function as defined by:

4.6.1 AGC > 1500/m^2, platelet count > 100,000/mm^3.

4.6.2 Bilirubin < 1.5; creatinine ≤ 1,5 mg/dl or calculated creatinine clearance ≥ 60 ml/min.

4.6.3 No evidence of congestive heart failure, symptoms of coronary artery disease, serious cardiac arrhythmias or evidence of prior myocardial infarction.

4.6.4 Adequate pulmonary reserve. Pulmonary function will be performed if clinically indicated in consultation with the Department of Internal Medicine. An FEV_1 ≥ 2.0 liters or ≥ 75% of predicted for height and age will be considered as minimum acceptable criteria for patient entry. These have been the eligibility requirements for NCI sponsored trials using rIL-2/LAK. We do not however anticipate pulmonary or cardiovascular problems with the current dose levels of IP rIL-2 which is below the MTD for rIL-2 (Chiron-CETUS) by the IP route.

4.7 No evidence of active infection which requires antibiotic therapy. Patients may become eligible after the infection is controlled.

4.8 No contraindication to the use of pressor agents.

4.9 Patients must have recovered from toxic effects of prior therapy. At least 4 weeks should have elapsed from prior chemotherapy or hormones and at least three months from radiation therapy before treatment with TIL.

4.10 Patients must be negative for HIV antibody by ELISA (or negative by Western blot if ELISA is positive) and negative for HB_s Ag.

4.11 The patient must give signed informed consent prior to the initiation of therapy or obtaining samples.

4.12 Exclusion Criteria

4.12.1 Patients who have extensive adhesions preventing free flow of fluid.

4.12.2 Intestinal dysfunction or uncorrectable intestinal obstruction due to disease or adhesions.

4.12.3 Patients with clinical disease who are good candidates for carboplatin reinduction.

4.12.4 Prior therapy with IP IL-2.

4.12.5 Prior abdominal irradiation (small field or pelvic RT alone may be eligible).

4.12.6 Any contraindication to surgical re-exploration.

4.12.7 Patients with organ allografts.

4.12.8 Patients who require or are likely to require cortiocosteroids for intercurrent disease.

4.12.9 A history of another malignancy other than basal cell carcinoma, or squamous carcinoma of the skin, or less than 10 years elapsed since remission from other malignancies.

4.12.10 Patients with any significant medical disease other than the malignancy. Patients with a major psychiatric illness are usually ineligible. Similarly, patients with unstable blood pressure, known CNS or seizure disorders, or severe diabetes mellitus will be ineligible. Patients with active infection are ineligible.

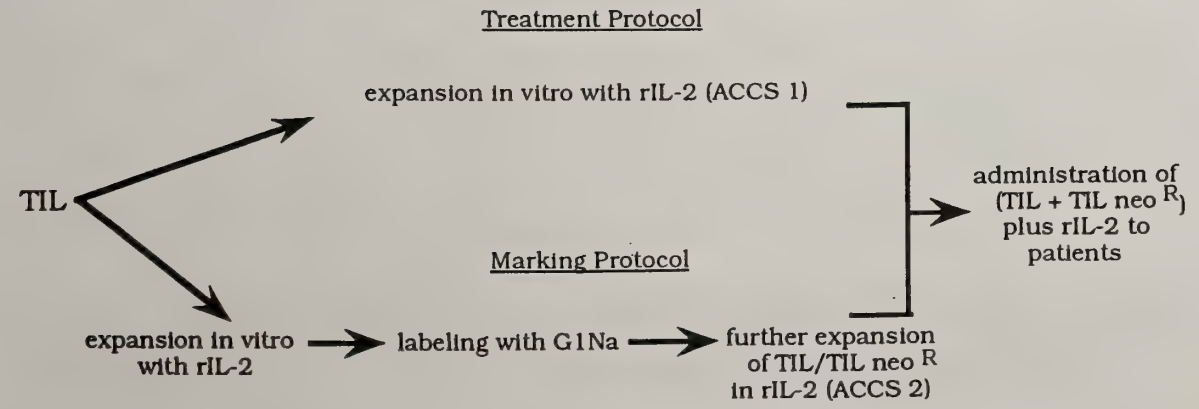
4.12.11 Patients with known coronary artery disease, myocardial disease, or significant valvular heart disease. Patients with documented significant ventricular ectopy. Patients who are suspected of having any one of the above may be eligible, but only after thorough cardiac evaluation.

5.0 RESEARCH PLAN

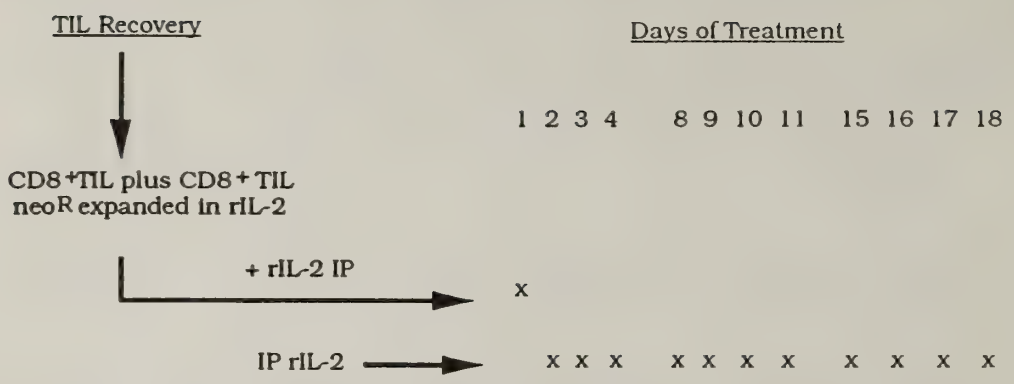
5.1 Registration
All patients will be registered with the Patient Data Management System of M. D. Anderson Cancer Center (713) 792-6828 (Wanza Scott, R.N.).

5.2 **(a) Schema for labeling of TIL with neo^R and treatment of ovarian cancer patients with intraperitoneal rIL-2 expanded purified CD3$^+$CD8$^+$ TIL and low dose rIL-2 (from Treatment Protocol ID92-015 (T92-0093). All patients who receive the retroviral marked TIL must also be eligible and receive treatment under protocol ID92-015 (T92-0093). The treatment and evaluation of clinical response to the treatment is described in protocol ID92-015 on pp. 14-20.**

Expansion and Marking of CD8$^+$ TIL with neo^R


Treatment Protocol
expansion in vitro with rIL-2 (ACCS 1)
TIL
administration of (TIL + TIL neo R) plus rIL-2 to patients
Marking Protocol
expansion in vitro with rIL-2
labeling with G1Na
further expansion of TIL/TIL neo R in rIL-2 (ACCS 2)

Treatment Schema


TIL Recovery
Days of Treatment
1 2 3 4 8 9 10 11 15 16 17 18
CD8+TIL plus CD8+ TIL
neoR expanded in rIL-2
+ rIL-2 IP
x
IP rIL-2
x x x x x x x x x x x

5.3 General Considerations

5.3.1 A temporary removable catheter will be placed under fluoroscopic guidance by an interventional radiologist.

5.3.2 Patients with major loculation will be excluded at this time.

5.3.3 Patients receiving TIL will be admitted for a minimum of 24-48 hours following I.P. TIL. In subsequent rIL-2 cycles patients may be treated as outpatients.

5.4 rIL-2 Administration

5.4.1 The first dose of rIL-2 (0.033 mg = $6x10^5$ IU) will be administered in the same bag as the TIL in D5 1/4 NS containing 0.1% albumin, USP. rIL-2 must first be dissolved in sterile USP water before dilution in the saline albumin solution.

5.4.2 rIL-2 will be given subsequently at a daily bolus dose of 0.033 mg/m^2 ($6x10^5 m^2$ IU) on days 2-4, 8-11, 15-18. All rIL-2 administered to patients will be prepared in 250 ml D5 1/4 NS + albumin 0.1% USP.

5.4.3 One to two liters of fluid (depending on patient size and comfort) will be maintained in the abdominal cavity to allow for adequate distribution of TIL + rIL-2.

5.4.4 Patients whose TIL do not meet the criteria for the trial will be taken off study.

5.4.5 During the administration of rIL-2, patients will be treated with acetaminophen at a dose of 650 mg q 4-6 hours. Non-steroidal anti-inflammatory drugs may be added if necessary. In addition, Carafate will be administered before meals and at bedtime and at least 4 times a day. Maalox can be added if necessary. The concurrent use of steroids is not allowed. If the patient develops severe chills and fever despite this premedication, Benadryl or Demerol in a dose of 25 mg - 50 mg I.V. may be used q 4-6 hours.

5.4.6 While in the hospital receiving TIL + rIL-2, patients will receive I.V. fluids as dictated by the patient's fluid needs. Since hypotension may occur with rIL-2 therapy, I.V. fluid rate will be increased if the systolic blood pressure drops below 90 mm Hg and the rate will be titrated to maintain the blood pressure

between 80 mm-90 mm Hg. In the unlikely event that hypotension develops to a level below 80 mm Hg and is not responsive to an initial challenge of I.V. fluids, it will be treated with low dose dopamine administration at a rate of 3-5 mcg/kg/minute.

5.5 Administration of TIL

5.5.1 An attempt will be made to administer the maximum TIL number of 10^{11} cells. A minimum number of $1x10^{10}$ cells will be acceptable.

5.5.2 TIL will be suspended in 300-500 ml D5 1/4 NS for injection and 0.1% albumin. rIL-2 at a dose of .033 mg is added to the saline-albumin solution prior to administration.

5.5.3 An initial infusion of approximately 10^8 cells in the saline solution will be performed followed by the remainder of the cells over approximately 20 - 60 minutes (with constant mixing of bag contents).

5.5.4 No filters are used in the infusion line.

5.5.5 Vital signs will be obtained before the infusion, every 15 minutes during the infusion, 30 minutes after the end of the infusion and then resumed every 4 hours. An emergency cart will be available at the bedside during and for one hour following administration of TIL + rIL-2.

6.0 PATIENT EVALUATION

The clinical and laboratory studies that are pertinent to the therapy protocol ID92-015 are described on pages 14-20.

Tumor and tissue biopsies, PBL and peritoneal cells will be obtained according to the schedule shown below.

Schedule for Samples During Treatment Course

Day	1	2	3	4	8	9	10	11	15	16	17	18	1 mo.
IP treatment													
(i) TIL	X												
(ii) rIL-2	X	X	X	X	X	X	X	X	X	X	X	X	
Tissue samples													
Tumor													X
Normal peritoneum													X
Omentum													X
Lymph node													X
Peripheral blood				X				X				X	X
Peritoneal fluid				X				X				X	X

7.0 EVALUATION AND MANAGEMENT OF TOXICITY

All toxicity will be graded. Previous unknown or severe toxicities must be reported to the NCI (Section 11.0).

8.0 CRITERIA FOR RESPONSE

Clinical response criteria are described in the treatment protocol ID92-015 (T92-0093).

The presence of G1Na transduced cells in tumor tissues and control sites will be detected by PCR using neo specific primers as described in Section 2.8 and Appendix 1 utilizing an improved PCR and Southern analysis procedure that included ^{32}P specific neoprobe.

8.1 In successful experiments a significantly increased neo^R signal detection by PCR and Southern analysis should be observed in tissues obtained at tumor sites. These results will be compared in biopsy material from normal tissues (peritoneum, omentum and lymph node tissue). To control for the presence of neo^R marked cells in blood, samples of blood will be obtained concurrently for PCR analysis. (See 10.0 Statistics Section for analysis of data).

8.2 The fold of enrichment of transduced cells at tumor sites will be determined by PCR performed on DNA extracted from tumor and nontumor tissue samples. The DNA will be extracted by proteinase K/phenol or Quagen (midi) DNA kit for blood and peritoneal fluid samples and analyzed by PCR. PCR quantitation will be performed on at least three replicates and results compared against the K562 neo^R standard. Extracted DNA samples will also be run in several dilutions.

8.3 In addition to these objectives we will assess the persistence of neo^R marked cells in the abdominal cavity we will obtain samples from the peritoneal cavity (through the catheter during the 18 days of rIL-2 administered) and washings obtained at laparoscopy.

9.0 CRITERIA FOR REMOVAL FROM STUDY

9.1 Progressive Disease.

9.2 Intercurrent illness which prevents further treatment.

9.3 Grade IV toxicity in the absence of a significant response which in the judgment of the investigator does not warrant the risk of further treatment.

9.4 Decision of the patient to withdraw from the study.

9.5 General or specific changes in the patient's condition which would render further rIL-2 therapy unacceptable treatment in the judgment of the investigator.

10.0 STATISTICAL CONSIDERATIONS

A justification is provided for undertaking a pilot study of 9 patients. It will be demonstrated that it is virtually certain that some lymphocytes sampled from each patient will be marked with the neo^R gene. In Dr. Rosenberg's study (37) uptake of neo^R marked TIL were demonstrated in malignant melanoma after patients received IV injections of neo^R transduced and nontransduced TIL where the proportion of neo^R transduced cells were as low as 1% or $60\text{-}200 \times 10^6$ neo^R marked cells. In our studies TIL will be administered by the IP and not the IV route. Therefore according to Rosenberg's results (37) if we inject only 100×10^6 neo^R transduced TIL we will be able to detect these cells in vivo. We anticipate that at least 10^{10} $CD8^+$ TIL can be injected into each patient. Of these 10^{10} $CD8^+$ cells at least half (5×10^9) will be from the neo^R infected bioreactor. With a neo^R transduction efficiency of 10% (as we have observed) 5×10^8 (10% of half 10^{10}) of the $CD8^+$ TIL will be marked. Hence, 5% (5×10^8 out of 10^{10}) of the injected cells will be marked with neo^R gene. We have assumed that a minimum of 10^{10} $CD8^+$ TIL can be injected into each patient. Of these 10^{10} $CD8^+$ TIL cells, only half (5×10^9) will be from the neo^R infected culture. A minimum neo^R transduction efficiency of 10% has been assured so that a minimum of 5×10^8 (10% of half of

after injection, will be $5x10^{8}/10^{12} = 5x10^{-4}$. Our detection methods employing PCR have a sensitivity of $1x10^{-5}$ cells so this procedure would be capable of detecting any marked cells that are among the sample sites under the null hypothesis that the $CD8^{+}$ TILs are homogeneously distributed after injection. Assuming a 0.1 gram tissue sample contains a approximately 10^{5} $CD8^{+}$ TIL (1% $CD8^{+}$ T cells), the expected number of marked cells is at least 50, since we have argued that the proportion of marked lymphocytes in a patient is $5x10^{-4}$ if they were homogeneously distributed. It is reasonable to assume that the number of cells marked in each tissue sample follows a Poisson distribution with a mean of 50. This means that the probability of observing at least one marked cell exceeds .9999 and if no marked cells are detected, the 95% confidence interval for portion of cells marked at that site is (0 to $3x10^{-5}$). Hence, the probability of a false negative is extremely low for each patient.

In the event that there is specific uptake neo^{R} marked TIL will not be equally distributed and substantial numbers will home into the tissue, much higher than those anticipated in normal tissues (postulated at 50 neo^{R} marked TIL/0.1 gm). Therefore with the PCR detection technique that we have established, it would be easy to identify and quantitate neo^{R} marked TIL that home to the tumor. PCR quantification in tumor and normal control sites will be performed according to the analytic methods of Morgan et al. (43). A successful outcome in this experiment is defined when the mean number of marked cells in tumor tissue is significantly larger than the mean number of marked cells in either the blood and the normal tissue. The mean referred to here is the average of the measurements taken in triplicate. If the first five patients exhibit a successful response, then there is significant evidence at the .05 level to reject the null hypothesis that the response rate is less than or equal to 1/2. In other words, if the first five patients exhibit a successful response, then there is evidence at the .05 level of significance that the success rate is greater than 1/2, and this part of the clinical trial can be terminated. In order to collect evidence that the probability of success is less than .30, 9 evaluable patients are needed. If all 9 patients have an unsuccessful outcome; i.e., the mean number of marked cells in tumor tissue is less than or equal to the mean number of marked cells in both the blood and the normal tissue, then there is evidence to reject the null hypothesis that the probability of success is greater than or equal to .30. That is, if all 9 patients have an unsuccessful outcome, then at the .05 level there is evidence that the probability of success is less than .30.

11.0 POTENTIAL SIDE EFFECTS AND REPORTING OF ADVERSE REACTIONS

11.1 Reporting of Adverse Reactions

Adverse drug reactions (ADRs) to G1Na neo^{R} are to be reported promptly to the Investigational Drug Branch. ADR reports are required even if there is only a suspicion of a drug effect. If in doubt call the Investigational Drug Branch of the NCI at 301-230-2330.

11.1.1 Adverse reactions are to be reported to the NCI in writing using the "NCI Adverse Reactions Form for Investigational Agents" appended to ID92-015 (T92-0093) within 10 working days.

11.1.2 Grade 4 reactions and patient deaths while on treatment are to be reported to NCI by phone within 24 hours. A written report is to follow within 10 working days.

11.1.3 Written reports should be sent within 10 working days to:

Investigational Drug Branch
Cancer Therapy Evaluation Program
P. O. Box 30012
Bethesda, MD 20824

and

Office of Recombinant DNA Activities
National Institutes of Health
Building 31, Room 4B11
Bethesda, MD 20892

11.1.4 All adverse reactions should be reported to the Institutional Review Board.

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THE UNIVERSITY OF TEXAS
M.D. ANDERSON
CANCER CENTER

Informed Consent

PROTOCOL TITLE: Use of a retroviral vector to study the trafficking patterns of purified ovarian tumor infiltrating lymphocytes (TIL) used in intraperitoneal adoptive immunotherapy of ovarian cancer patients. A pilot study

1. _____ _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other informed consents you may have signed in other protocols.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY: This is a clinical research study to determine whether lymphocytes (white blood cells) that are cultured from a patient's tumor, migrate back to the tumor after injection. Patients who participate in this marker study must also be participants in the companion therapy trial (ID92-015).

3. DESCRIPTION OF RESEARCH: Lymphocytes that are obtained from tumors are called tumor infiltrating lymphocytes or TIL. These TIL are grown in culture together with interleukin-2 before being administered intraperitoneally to patients in the accompanying therapy protocol ID92-015. Patients whose TIL could not be grown in culture and those with medical immunologic disorders which would make them unsuited for treatment, are excluded from the trial. During the growth of the TIL in culture a bacterial "marker" gene will be inserted into the TIL using a defective mouse retrovirus so that they can be traced after reinjection into the patient. Samples of blood (about 10 cc each) and peritoneal fluids (approximately 100 cc) will be obtained from the patient for treatment with TIL and on days 4, 11, 18 and at one month after receiving the TIL injection. One month after receiving the TIL, patient's will undergo a laparoscopy to evaluate their responses to the treatment. At this time biopsies will be obtained from tissues suspected of being involved with cancer. Biopsies will also be obtained from certain normal tissues, e.g., peritoneum (lining of abdominal cavity) and lymph node (if easily accessible).

These biopsies will be obtained to determine if the TIL have migrated to the tumor. This information might be useful for the development of future treatment strategies in other patients with ovarian cancer. The laparoscopic surgery will be performed to evaluate the response to the treatment and not for the marker study alone. There is no cost to the patient for the preparation of the cells for the treatment or for the retrovirus marker. There is also no cost to the patient from special laboratory tests related to the conduct of this protocol. Patients will be followed indefinitely and if the patient happens to move, a forwarding address will be requested. In the unfortunate event of death, an autopsy will be requested from family members. Nine patients may receive retrovirus-modified cells in this protocol.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

This virus and another like it have been given to over 100 patients for at least the past 4 years at other institutions. No adverse effects were observed in these studies because the virus is modified so that it cannot cause an infection in the cells of the body.

Risks, however, which may not yet have been observed, are possible. These are "theoretical risks" since they have not yet been seen in patients who have received the retrovirus. It is possible that the virus could change the cells which are marked so as to grow in an abnormal pattern, and even cause cancer or leukemia. The gene marker might also produce a protein which might inactivate certain antibiotics but alternative antibiotics are available so that this does not constitute a risk or threat for patients during therapy.

There is a small risk of bleeding from biopsy sites.

This clinical research study may involve unforeseeable risks to the participant.

5. POTENTIAL BENEFITS: There are no direct therapy benefits to the patient whose TIL have been marked with the gene containing the retrovirus. Information provided may be able to show that the TIL have a preferred uptake at tumor sites.

6. ALTERNATE PROCEDURES OR TREATMENTS: The participant may receive the TIL in the treatment protocol without these cells being exposed to the retrovirus.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the procedure involved and the investigator has been willing to reply to my inquiries. This procedure will be administered under the above numbered, title, and described clinical research protocol at this institution. I hereby authorize Dr. Ralph S. Freedman the attending physician/investigator and designated associates, to administer this procedure.

8. I have been told and understand that my participation in this clinical research study is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

   Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or effects of my decision with my physician.

   In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

   I will be informed of any new findings developed during the course of this clinical research study which may relate to my willingness to continue participation in this study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the National Cancer Institute, Food and Drug Administration, and Genetics Therapy Incorporated, and Applied Immune Sciences, Inc., may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available for treatment at this institution. I understand, however, that I cannot expect to receive any credit or

reimbursement for expenses from this institution or any financial compensation from this institution for such injury.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of this study, I understand that I may be required to cover the cost of subsequent doses.

    Costs related to my medical care including expensive drugs, tests or procedures that may be specifically required by this clinical research study shall be my responsibility unless the sponsor or other agencies contribute toward said costs. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, devices, new drugs, or possible patentable procedures, in which event I understand that I cannot expect to receive any compensation or benefits from the subsequent use of information acquired and developed through my participation in this research project.

13. I may discuss questions or problems during or after this study with Dr. Ralph S. Freedman at (713) 792-2770. In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman of the Surveillance Committee at (713) 792-3220 and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT:

Based on the above, I consent to undergo the described procedures and investigational therapy and have received a copy of the consent form.

_____	_____
DATE	SIGNATURE OF PARTICIPANT
_____	_____
WITNESS OTHER THAN PHYSICIAN INVESTIGATOR	SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and I believe the participant understood this explanation.

_____
PHYSICIAN/INVESTIGATOR

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME	POSITION TITLE
Ralph S. Freedman, M.D., Ph.D.	Professor of Gynecology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Witwaterstrand University (Johannesburg)	M.B. Ch.B.	1965	Medical
Witwaterstrand University (Johannesburg)	Ph.D.	1975	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1971 - Royal College Obstetricians and Gynecologists (M.R.C.O.G.)

1976 - 1977 Eli Lilly - International Fellowship in Immunology under Joseph Sinkovics, M. D. Anderson Cancer Center, Houston, TX.

1979 - 1986 Associate Professor and Associate Surgeon, U.T. M.D. Anderson Cancer Center, Houston, TX.

1980 - Fellow American Board Obstetrics and Gynecology (F.A.C.O.G.)

1984 - Fellow American College of Surgeons

1984 - 1990 Vice Chairman Surveillance Committee, U.T. M.D. Anderson Cancer Center, Houston, TX.

1987 - 1988 Vice Chairman Research Committee, U.T. M.D. Anderson Cancer Center, Houston, TX.

1987 - Present Member - Department of Clinical Immunology, Biologic Response Sciences, Ph.D. Program in Cancer Biology, Houston, TX.

1987 - Present Professor of Gynecology and Surgeon of Gynecologic Oncology, U.T. M.D. Anderson Cancer Center, Houston, TX.

1988 - Present Chief, Section of Experimental Gynecology, U.T. M.D. Anderson Cancer Center, Houston, TX.

1988 - Present Regular Member - Faculty of Immunology, Graduate School of Biomedical Sciences, Houston, TX.

1989 - Present Co-Chairman, Surveillance Committee, U.T. M.D. Anderson Cancer Center, Houston, TX.

CONSULTANT

Lead Reviewer
1993 Special Review Committee, National Cancer Institute on (RFA) CA-92-28 Clinical trials of cancer therapy with BRMs.

Special Consultant
1993 National Federation of Cancer Centers of France (Equivalent of the National Cancer Institute).

CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

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HUMAN GENE TRANSFER PROTOCOL

9406-076 Heslop, Helen; Brenner, Malcolm, K.; and Krance, Robert; St. Jude Childrens Research Hospital, Memphis, Tennessee; *Use of Double Marking with Retroviral Vectors to Determine the Rate of Reconstitution of Untreated and Cytokine Expanded CD34(+) Selected Marrow Cells in Patients Undergoing Autologous Bone Marrow Transplantation.*

Date of RAC Approval: 6-9-94
Date of NIH Approval: 7-12-94

POINTS TO CONSIDER

Protocol: Use of double marking with retroviral vectors to determine rate of reconstitution of untreated and cytokine expanded CD34+ selected marrow cells in patients undergoing autologous bone marrow transplantation

SCIENTIFIC ABSTRACT

Autologous BMT in is frequently used as consolidation or salvage therapy in pediatric malignancy to allow administration of intensive chemotherapy where hemopoietic suppression would otherwise be dose limiting. This increased dose of chemotherapy and radiation therapy may allow a higher proportion of patients to be cured than would be possible with conventional therapy. One of the major causes of morbidity after autograft is the risk of infection and bleeding during the period of pancytopenia which follows the administration of ablative chemotherapy. One way of improving autologous stem cell rescue is therefore to shorten the period of aplasia which follows the procedure and thereby reduce the morbidity and mortality. A second improvement would be to harvest as few stem cells as possible. One way to achieve both these aims is by expanding committed and uncommitted progenitor cells ex vivo.

We and other investigators have evaluated the ability of growth factors to expand CD34 enriched marrow cells ex-vivo and noted little expansion of the most primitive cell populations (appendix A). While growth factor treated marrow may therefore produce earlier engraftment, its capacity to produce long term reconstitution remains unknown. There is a concern that the use of more extensive growth factor combinations, whose activities reached further back in hemopoiesis, would differentiate rather than expand or maintain true stem cells. If so, such marrow would produce earlier reconstitution but at the expense of being unable to contribute to long term engraftment

The ideal of ex vivo expansion is to increase both committed and non-committed progenitor cells thereby accelerating initial engraftment and ensuring sustained reconstitution. An acceptable alternative is simply to expand committed progenitor cells to reduce the period of post transplant aplasia provided sufficient pluripotent stem cells were retained in an undifferentiated state to permit long term recovery. Gene marking of marrow ex vivo allows determination of whether adequate numbers of stem cells remain in vivo. In this study we plan to use genetic marking of marrow ex vivo with two distinct vectors to compare the subsequent in vivo reconstitution of two aliquots of CD34 selected marrow - one treated and one unmanipulated. This protocol will therefore answer questions about the feasibility of accelerating hemopoietic reconstitution and the impact of such action on uncommitted progenitor cells. It will be a generic processing and transduction protocol for patients receiving autologous BMT for pediatric malignancy.

NON-TECHNICAL ABSTRACT

Autologous bone marrow transplant is often used to allow higher doses of chemotherapy to be given to patients with pediatric cancer. One of the risks of this treatment is that patients are at risk of infection and bleeding until the new marrow has grown back. One approach to improving the results of such transplants is to preincubate the marrow with growth factors so that the infused marrow takes more quickly. If treated marrow takes more quickly we may also be able to harvest smaller amounts of marrow in the future. To determine if treatment of marrow with growth factors does lead to faster take and also to see if these cells persist long term, we plan to compare recovery of two portions of marrow cells; one treated with growth factors and one untreated.

To do this we will mark these two portions of marrow with the neomycin resistance gene in two distinguishable vectors. This will allow us to learn how quickly each portion engrafts and how long each portion survives in the patient. This information will allow us to learn if this approach may be beneficial in shortening aplasia following autologous BMT and thereby reduce the risks of the procedure.

SJCRH
2/94;3/94;3/30/94

USE OF DOUBLE MARKING WITH RETROVIRAL VECTORS TO DETERMINE RATE OF RECONSTITUTION OF UNTREATED AND CYTOKINE EXPANDED CD34+ SELECTED MARROW CELLS IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION

PRINCIPAL INVESTIGATORS
Helen E. Heslop, MD, FRACP, FRCPA
Malcolm K. Brenner, PhD, FRCP
Robert A Krance MD
Division of Bone Marrow Transplantation

CO-INVESTIGATORS
Richard Heideman MD
Department of Hematology/Oncology

John M Cunningham, M.D
Division of Bone Marrow Transplantation

Stacye Richardson
Timothy York
Bone Marrow Processing/Graft Engineering

James M. Boyett, Ph.D.
Deo-Kumar Srivastava, Ph.D.
Department of Biostatistics

St. Jude Children's Research Hospital
332 N. Lauderdale, P.O. Box 318
Memphis, Tennessee 38101-0318
(901) 522 0300

Ronald Berenson, MD
CellPro Inc
Bothell WA

Stephen G. Marcus, MD
Genetic Therapy, Inc.
Gaithersburg, MD

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APPENDIX A: Pre-Clinical Data on Selection and Ex Vivo Expansion of CD34 Cells
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1.0 CHECKLIST FOR PATIENT ELIGIBILITY PRIOR TO BMT

NO	YES	
___	___	Age less than 21 years at time of diagnosis
___	___	Receiving unpurged autologous BMT for therapy of leukemia or solid tumor

To check eligibility call Dr. Heslop at 531-2529 or Dr. Krance at 522-0336.

___	___	Informed consent explained to and signed by patient/guardian
___	___	Check table in Section 8.2 of this protocol to see necessary baseline and follow-up studies

2.0 OBJECTIVES

2.1 To compare hemopoietic reconstitution (time to 100 neutrophils, time to 500 neutrophils and persistence of engraftment) of untreated and ex-vivo expanded aliquots of CD34 selected cells in patients receiving unpurged autografts as therapy for pediatric malignancy.

2.2 To evaluate the safety of ex vivo treatment of marrow with growth factors.

3.0 BACKGROUND AND RATIONALE

3.1 Use of autologous BMT as therapy for pediatric malignancy

The use of autologous BMT in pediatric malignancy either as consolidation therapy in high risk patients or as salvage therapy after relapse is increasing.[1-4] The underlying rationale for this approach is that the availability of cryopreserved marrow for reinfusion allows the administration of intensive chemotherapy where hemopoietic suppression would otherwise be dose limiting. This increased dose of chemotherapy and radiation therapy may allow a higher proportion of patients to be cured than would be possible with conventional therapy.

Despite this increasing utilization the biology of autologous BMT has been poorly understood. It was unknown for example whether the procedure merely provided a temporary source of hemopoietic cells until hemopoiesis was re-established from residual recipient cells. In previous studies in which the harvested bone marrow from patients with AML or neuroblastoma was marked with a neomycin resistance gene, we have shown that the marker gene continues to be detected for up to two years post BMT. This result demonstrates that the infused marrow does indeed contribute to sustained long term

engraftment.[5] In these studies, marrow mononuclear cells were incubated with retroviral supernatant for 6 hours in the absence of growth factors.

3.2 Strategies for accelerating Engraftment

One of the major causes of morbidity after autograft is the risk of infection and bleeding during the period of pancytopenia which follows the administration of ablative chemotherapy. Several strategies have been employed to try and accelerate reconstitution including administration of growth factors in vivo to patients following transplant,[6] the use of peripheral blood stem cells as well as marrow[7] and incubation of marrow with growth factors. While incubation of normal marrow with lineage restricted growth factors may expand committed progenitor cells and modestly accelerate recovery in allograft recipients,[8] it is unclear if this approach would be successful in autograft patients whose marrow has been extensively exposed to myeloablative agents. Moreover, there is a concern that the use of more extensive growth factor combinations, whose activities reached further back in hemopoiesis, would differentiate rather than expand or maintain true stem cells. If so, such marrow would produce earlier reconstitution but at the expense of being unable to contribute to long term engraftment

For example in studies at the National Institutes of Health, CD34 selected cells were transduced in the presence of IL6, IL3 and SCF for 72 hours, in an attempt to enhance transduction of early and committed progenitor cells.[9] In fact in these studies, the marker gene was subsequently detected in vivo at lower levels and for a shorter period than had been observed in the St. Jude studies where unmanipulated marrow is marked (Cindy Dunbar, personal communication). While such observed differences may be due to differing intensity of conditioning regimens and underlying malignancies it is possible that the presence of growth factors in the culture may result in the loss of pluripotent stem cells which are driven instead to differentiation.

3.3 Use of CD34 selected cells for stem cell rescue

CD34 is a 115kD glycoprotein present on 0.5-3% of bone marrow cells comprising the most immature subset including the majority of progenitors assayed in clonogenic assays. It is not expressed on mature hemopoietic progenitors nor on the surface of non-hemopoietic pediatric tumor cells. The use of CD34 selected marrow has several theoretical advantages in autologous BMT. First it may serve as a purging procedure as malignant cells not expressing CD34 will not be selected and cryopreserved. Secondly such selection allows reduction in the number of cells cryopreserved and reinfused with consequent reduction in morbidity of reinfusion. Finally in gene marking protocols CD34 selection reduces the volume of clinical grade supernatant required for transduction.

Clinical studies in patients receiving autografts for neuroblastoma or breast cancer used CD34+ marrow or peripheral blood cells selected on the Cell Pro column which utilizes an immunoadsorption technique.[10,11] Cells are incubated with biotinylated anti-CD34

monoclonal antibody and then passed through a column of avidin-coated beads. Cells binding antibody are retained due to the high affinity between avidin and biotin. Bound cells are eluted from the column by mechanical agitation of the beads which disrupts the CD34 antibody interaction.

The purity of the selected population ranged from 35-95% with a CFU-C enrichment of 22-180 fold. Patients received 0.4-3 x 10^6 CD34 cell/kg. In the breast cancer study the median time to an ANC of 500 was 20 days in patients receiving CD34 selected marrow without post transplant growth factors and 11 days in patients treated with GCSF post transplant.

3.4 Ex vivo expansion of CD34 selected cells

Autologous stem cell rescue has two apparently conflicting aims - a short period of aplasia with harvesting of as few stem cells as possible. One way to achieve both these aims is by expanding committed and uncommitted progenitor cells ex vivo. Several investigators have evaluated the ability of growth factors to expand CD34 enriched marrow cells ex-vivo.[12-14] Brugger et al[13] have shown that the combination of IL1,, IL6, SCF, Erythropoietin and IL3 results in a 260 fold expansion in total cell numbers, and a 3 fold increase in CD34 cells. We have obtained similar results in our preclinical studies (Appendix A). We and others have, however, noted little expansion of the most primitive cell populations, so that while treated marrow might produce earlier engraftment, its capacity to produce long term reconstitution remains unknown.

3.5 Use of gene marking to compare reconstitution

The ideal of ex vivo expansion is to increase both committed and non-committed progenitor cells thereby accelerating initial engraftment and ensuring sustained reconstitution. An acceptable alternative is simply to expand committed progenitor cells to reduce the period of post transplant aplasia provided sufficient pluripotent stem cells were retained in an undifferentiated state to permit long term recovery. Gene marking of marrow ex vivo allows determination of whether adequate numbers of stem cells remain in vivo. In this study we plan to use genetic marking of marrow ex vivo with two distinct vectors to compare the subsequent in vivo reconstitution of two aliquots of CD34 selected marrow - one treated and one unmanipulated. This protocol will therefore answer questions about the possibility of accelerating hemopoietic reconstitution. It will be a generic processing and transduction protocol for patients receiving autologous BMT for pediatric malignancy.

3.6 Advantages of gene marking

One of the major difficulties in assessing the impact of growth factors on marrow recovery is the wide interpatient variability. This may be further compounded by differences in marrow involvement with tumor, pre-transplant therapy and in conditioning

regimens used. One of the most important potential advantages of the double gene marking approach is that each patient acts directly as their own control for the effects of growth factors, greatly increasing the power of the analysis on even a limited number of patients. This issue is addressed in the Statistical Section. Because each patient will act as their own control differences in underlying diseases and the degree of ablation the conditioning regimen will induce are not relevant.

4.0 STUDY OUTLINE

Marrow will be harvested as patients recover from previous chemotherapy. At least 3 x 10^8 cells/kg will be harvested. 10^8 cell/kg will be frozen as a safety backup and the remaining cells will be processed on the CellPro column to purify CD34 cells. The purified CD34 population will be split into two aliquots which will randomly be marked with the LNL6 or G1Na vector. One aliquot will be frozen without further manipulation while the remaining fraction will be incubated in the presence of growth factors for 5-7 days and then frozen. Patients will be conditioned with the regimen specified on their disease based protocol and both aliquots of CD34 cells will then be administered simultaneously at the time of transplant. We will use semiquantitative PCR to detect the presence of both marker genes in patients blood and marrow at various intervals post transplant.

Outline of Protocol

1/3 marrow Safety backup	2/3 marrow CD34 selection	
	Mark with LNL6	Mark with G1Na
	Cryopreserve or Expand ex vivo with growth factors	Expand ex vivo with growth factors or Cryopreserve
	Reinfuse both aliquots at time of BMT	

5.0 STUDY DESIGN

5.1 Patient Eligibility

To ensure sufficient cells are present in the CD34 selected fraction as well as in the backup marrow at least 3 x 10^8 cells/kg must be harvested. If the yield is less than this number either a second harvest must be performed or the patient is not eligible for this study.

5.2 Marrow Processing

Details are provided in Appendix B. In brief mononuclear cells are obtained using the cell processor. 1/3 is frozen immediately without further manipulation and the remaining 2/3 is processed on the Cell Pro column to select CD34 cells as described in Appendix B.

5.3 Transduction with the neomycin resistance gene

CD34 selected cells are transduced with the G1Na or LNL6 vector, used in the previous St. Jude gene marking protocols, AMLREM, NEBREL, NEBREM, PAMMYLA and ETNA as described in Appendix C. The efficiency of marking will be estimated by clonogenic growth in G418 and semi-quantitative PCR for the neomycin resistance gene.

5.4 Incubation with growth factors

After marking one aliquot will be frozen immediately while the other aliquot will be incubated with the growth factors IL6, IL3 and SCF for 5-7 days as described in Appendix D.

6.0 PATIENT ELIGIBILITY (See Section 1.0)

6.1 All patients receiving an unpurged autologous BMT will be eligible for this protocol including:-

6.1.1 Patients enrolled on the ABMT4 study or receiving unpurged marrow on the NB91 study (Dr Santana Principal Investigator)

6.1.2 Patients receiving autografts on CNS 15 as part of therapy for brain tumors (Dr Heideman Principle Investigator)

6.2 Exclusion Criteria

6.2.1 Patients receiving autologous BMT with marrow purging on the PAMMYLA or BANG protocols

6.2.2 Patients on whom the harvest yield $< 3 \times 10^8$ cells/kg.

7.0 TREATMENT PLAN

The treatment plan and supportive care is described in the primary autograft protocol specific for the patient's malignancy.

8.0 PATIENT EVALUATION

8.1 Regeneration of Cryopreserved Autologous Marrow

In the absence of engraftment at 30 days (Neutrophils $< 100/mm^3$) patients will receive a 10 course of G-CSF. If ANC remains $\leq 100/mm^3$, the reserve, unmanipulated marrow aliquot will be returned. The reserve marrow may be returned earlier in the presence of significant infectious complications after discussion with the P.I. of the protocol.

When marrow is obtained to assess engraftment and disease progression, additional cultures will analyze which marker genes are present in progenitor cells and in what lineages. We will note time to engraftment of 100 neutrophils and 500 neutrophils and the contribution of each aliquot of marrow at these times, using semiquantitative PCR. Patients will also be monitored at weekly intervals for 12 weeks, monthly intervals for 6 months and annually thereafter for 5 years for the long-term persistence of cells containing the marker genes in the peripheral blood (PB) using FACS sorted populations. PB will be separated into T cells, B cells, monocytes and granulocytes using appropriate monoclonal antibodies (MAb), and each lineage examined for the proviruses. Again, clonogenic analyses of progenitor cells grown in methylcellulose will be used to compare the contribution of each marrow aliquot to reconstitution.

8.2 Other Studies.

8.2.1 The following investigations will be obtained pre infusion, then 3 x weekly for 6 weeks, then weekly for 6 weeks then monthly for 1 year.

CBC and diff

BUN,creatinine, bilirubin, SGOT,SGPT, alkaline phosphatase, Na, K, Cl, CO_2, albumin, total protein, glucose.

8.2.2 The following investigations for monitoring of retrovirologic safety will be obtained pre infusion, then monthly for 3 months, 3 monthly for 1 year and then yearly.

Peripheral Blood in preservative free heparin (20 ml for BMT lab). This blood will be used for the following studies.

Analysis for viral nucleic acids in PBLs by PCR.
Reverse transcriptase assay
Serology for retrovirus envelop protein

8.3. Other tissues

8.3.1 Autopsy will be requested if the patient dies

9.0 EVALUATION DURING STUDY

9.1 Follow-up Interval

Patients shall be seen at thrice weekly intervals at St. Jude Children's Research Hospital for 6 weeks, then weekly for 6 weeks then monthly for one year. Additional visits will be obtained as clinically indicated.

9.2 Potential Accrual and Study Duration

Fifteen to twenty patients will be eligible for this protocol per year so accrual for this first planned study should be complete in 18-24 months. We would then plan to substitute a follow up study using new cytokines or conditions for ex vivo expansion based on our preclinical studies

10.0 STATISTICAL CONSIDERATIONS

This study is proposed to investigate relationships between ex vivo exposure of CD34+ BM cells to growth factors and the times to engraphment following reinfusion of these cells and to loss of evidence of presence of cells derived from the cells incubated with the growth factors. The assumption is made that neither disease nor conditioning regimen will affect the results. There are concerns for the safety of the patients and these concerns are reflected in the design through monitoring of deaths and excessive toxicity with stopping rules described below.

Each patient will receive autografts of two sources of CD34+ cells. Initially two aliquots of approximately 10^8 marrow cells will be processed to select the CD34+ cells. One resulting aliquot will be transduced with the G1Na vector and the other with the LNL6 vector. One of these marked aliquots will be randomly selected to be incubated for 5-7 days with the growth factors IL1, IL6, IL3, and SCF and the other will be frozen. At the beginning of the study, sufficiently large batches of the two vectors will be obtained to permit the entire investigation to be accomplished with the same batches. The randomization is necessary to prospectively adjust for the possible differing transduction rates of each vector. For each patient the transduction efficiency of each vector will be recorded. After the incubation period the frozen aliquot will be thawed and both aliquots of cells reinfused into the patient. Estimates of the numbers of reinfused CD34+ cells will be recorded for each aliquot.

Weekly for 12 weeks following autograft, peripheral blood samples will be drawn to count the number of absolute neutrophils (AN) and assayed for the presence of each provirus. This can reliably be accomplished if the ANC exceeds 100. The date of the sample, ANC and proportions (p_{BG} and P_{BL}) of AN's containing the G1Na and LNL6 provirus, respectively, will be recorded for each sample even if the absolute neutrophil count is less than 100, as will time to engraftment, defined as an ANC greater than 500. Bone marrow aspirates will be available on many of the patients at several times following autografting. These samples, when available, will contribute accurate estimates of the proportion (P_{MG} and P_{ML}) of marrow cells which are marked with the G1Na and LNL6 vectors, respectively.

10.1 Modeling Time to Engraftment

The problem is to model the time to engraftment as a function of covariates and in particular, to describe the relationship between the proportion of AN's expressing each gene to this time. Censoring is possible. Our first approach will be based on the Cox Life Table Regression model (A). Fixed covariates to be investigated include transfection rates and numbers of CD34+ cells reinfused. Time dependent covariates to be investigated include time from autograft to ANC first exceeding 100, P_{BG}, and P_{BL}. The function of the two peripheral blood proportions, which is hypothesized to relate to the time to engraftment, is the rate of change of these proportions. For each time point following two estimates of a proportion, simple linear regression will be used to provide an estimate of these rates. If such an estimate is not possible at a particular point, zero will be used in the model as the observed rate. If we are successful in modeling the change in proportions over time, we will consider imputing the unknown values instead of using zero. Interactions between the two factors as well as with time will be included in the model. Other functions will be explored, but the rate of change is expected to contain the important information relative to time to engraftment. Other linear models to be explored will reflect that the growth of the proportion of cells marking with a gene may be exponential and a function of the transfection rates and numbers of cells reinfused. After establishing the model using the peripheral blood proportions, a subset

analysis will be performed for those patients for whom marrow samples are available to learn if these additional factors contribute to predicting time to engraftment. The least squares technique is used to summarize data repeatedly measured for each patient, and since no inference will be made on these estimates, the usual regression assumptions (which are not true in this case) of normally distributed independent errors are not required. The time dependent covariates will be recomputed at each successful engraftment. As time progresses, more and more weekly measurements will be available for estimation of the hypothesized functions to be included in the Cox model.

One significant difficulty with this approach is that the proportions will be estimated with error and the magnitude of that error is not known. Indeed, we will only know that a particular proportion lies between two numbers. We will investigate the effect of this uncertainty on our resulting model. Furthermore, no data presently exist which would permit reasonable statistical statements regarding confidence that the proposed approach will be successful or that the basic hypotheses will be tested with prescribed statistical power. Therefore, we propose to conduct a pilot study of 20 patients in order to estimate errors associated with the discussed estimates of important covariates and to the examine the hypothesized functions which could be used to model time to engraftment. After a thorough analysis, a second study will be designed to confirm the model and to test with adequate statistical power, the basic hypothesis that exposure to growth factors accelerates engraftment.

10.2 Modeling Time From Engraftment to Failure to Detect Cells Exposed to Growth Factors

Following engraftment, patients will be monitored as described in other sections of the protocol for the presence and proportion of AN contributed by cells generated by those cells exposed and not exposed to growth factors ex vivo. Similar to the above approach, we will model the time to loss of evidence of the cells of interest beginning from the date of engraftment. Loss will be established by two consecutive assays being negative.

10.3 Safety Monitoring

There is now considerable experience documenting the toxicity and failure rate of ABMT in this setting as well as the safety of the gene marking techniques. Nonetheless, we will sequentially monitor the study for an unexpectedly high frequency of these events.

Death during ABMT:

If four deaths during transplantation (first 100 days) are observed in the treatment of the first 20 patients, accrual to the study will be terminated and a complete evaluation of all information made as to whether continuation is warranted. Accrual will be halted when the fourth death is observed. Using the negative binomial distribution, one can show that this decision rule provides for terminating the study early with probabilities of 0.02, 0.13 and 0.35 if the true unknown death rate is 0.05, 0.10 or 0.15, respectively. If the true

unknown death rate is higher than the figures above, the design provides even higher chances of stopping the trial early for reevaluation. The probability for terminating accrual early is 0.59, 0.89 and 0.98 if the true death rate is 0.20, 0.30 or 0.40, respectively.

Toxicity

If six episodes of unacceptable (Grade IV) toxicity or death during BMT are observed in the first 20 patients treated, then accrual to the study will be terminated and all data evaluated to determine whether continuation is warranted. This decision rule provides for terminating the study early with probabilities of 0.01, 0.07 and 0.20 if the true unknown toxic rate is 0.10, 0.15 or 0.20, respectively. If the true unknown toxic rate is unacceptably large we will have an even greater chance of stopping the trial early for reevaluation. The probability for terminating accrual is 0.38, 0.58 and 0.87 if the true toxic rate is 0.20, 0.30 or 0.40, respectively.

11.0 RECORDS TO BE KEPT

Flow sheets documenting dates and doses of therapy as well as clinical chemistries, hematologic parameters, the clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

Imaging reports

Surgical summaries

Autopsy summaries, where appropriate

Date of entry on to other appended protocols

Informed consent documents

All required clinical evaluation records will be the responsibility of Drs Helen Heslop or Robert Krance who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of retroviral insertion will be the responsibility of Drs. Brenner and Heslop.

12.0. REPORTING REQUIREMENTS

12.1 Register all patients with Bone Marrow Transplant Data Manager at Ext .

12.2 Enter all patients by phoning Dr Heslop Dr Krance or Dr Brenner. The following forms should be completed:

On study form

Investigational agents flow sheet forms

Off study forms

12.3 Adverse reactions will be reported by phone to Drs Heslop, Krance or Brenner within 24 hours of their occurrence. These adverse reaction reports include all life-threatening events (Grade 3/4 toxicity) and all fatal events.

13.0 INFORMED CONSENT

All patents and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by him or his legal guardian. *Full confidentiality of patients and patient records will be provided according to institutional guidelines.*

14.0 REFERENCES

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2. Horowitz ME, Kinsella TJ, Wexler LH, Belasco J, Triche T, Tsokos M, Steinberg SM, McClure L, Longo DL, Steis RG, et al : Total-body-irradiation and autologous bone marrow transplant in the treatment of high risk Ewing's sarcoma and rhabdomyosarcoma. Journal of Clinical Oncology 11:1911-1918, 1993.

3. Vose JM, Armitage JO: Role of autologous bone marrow transplantation in non-Hodgkin's lymphoma. Hematol Oncol Clin North Am 7:577-590, 1993.

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5. Brenner MK, Rill DR, Holladay MS, Heslop HE, Moen RC, Buschle M, Krance RA, Santana VM, Anderson WF, Ihle JN: Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. Lancet 342:1134-1137, 1993.

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8. Naparstek E, Hardan Y, Ben-Shahar M, Nagler A, Or R, Muncuoglu M, Weiss L, Samuel S, Slavin S: Enhanced marrow recovery by short preincubation of marrow allografts with human recombinant interleukin-3 and granulocyte-macrophage colony-stimulating factor. Blood 80:1673-1678, 1992.

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12. Haylock DN, To LB, Dowse TL, Juttner CA, Simmons PJ: Ex vivo expansion and maturation of peripheral blood Cd34+ cells into the myeloid lineage. Blood 80:1405-1412, 1992.

13. Brugger W, Mocklin W, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L: Ex vivo expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, Interleukin-1 beta (IL1-beta), Il-6, IL-3, interferon-gamma and erythropoietin. Blood 81:2579-2584, 1993.

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PARENT'S/PATIENT'S SUMMARY STATEMENT
USE OF DOUBLE MARKING WITH RETROVIRAL VECTORS TO DETERMINE RATE OF RECONSTITUTION OF UNTREATED AND CYTOKINE EXPANDED CD34+ SELECTED MARROW CELLS IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION

You have consented for an autologous bone marrow transplant as part of the treatment for your/your child's cancer. In this procedure strong chemotherapy is given to kill any cancer cells that may be left. Then, a bone marrow transplant will be done using your (your child's) own bone marrow. This is necessary because the chemotherapy also kills normal cells in the marrow.

TREATMENT OF MARROW BY CHOOSING STEM CELLS AND USING GROWTH FACTORS WITH GENE MARKING

After autologous bone marrow transplant there is a risk of infection and bleeding while the blood counts are low before the new marrow grows back. In this study we would like to find out if we can make the marrow grow faster after we put it back by mixing it with special proteins called growth factors. To do this, we will split the marrow into three parts. One part will be frozen without treatment as a safety backup. The other part will be separated on a machine which picks out cells called stem cells which grow up to be normal marrow cells. We will split the stem cells into two parts and put a different marker gene into each of them. One marked part will then frozen without more treatment, and the second will be given growth factors to try and make the marrow grow faster. Both parts of the stem cells will then be returned to you/your child. We will then look to see if the treated part of the marrow grows back faster and stronger than the untreated part. Each step of the treatment plan is given below.

MARROW HARVEST AND MARKING

Before the high-dose chemotherapy starts, we will remove a small part of your/your child's bone marrow from the hip bone. This will be done in the operating room under general anesthesia. You/your child will not feel anything when the marrow is taken. There may be some pain later. You/your child will be given medicine for the pain.

If you agree (your child agrees) to be in this study, we will freeze about one-third of the marrow with no treatment. This bone marrow will be saved as "back up" bone marrow to be given to you (your child) just in case the first bone marrow does not take. Stem cells will be picked from the rest of the marrow and half will be marked with LNL6 and half with G1N. These are the names of special mouse viruses that have been changed to keep them from causing infection. The marker, a bacterial gene called "neoR", is put inside these special viruses. One half of the marrow will then be frozen immediately and the other will be cultured in growth factors for 5-7 days and then frozen. Both parts will then be put back through you/your child's central line after the chemotherapy is finished. The markers let us follow the treated marrow in your (your childs) body to see if treatment with growth factors makes the marrow grow back faster.

BENEFITS

The benefits of this study would be that we could learn whether if treating the marrow with growth factors makes it grow back faster and makes the risk of infection smaller and you/your child's hospital stay shorter. Stem cell section may also mean that only normal marrow cells and not cancer cells are given back to you/your child. This information might help you/your child. Otherwise, what we learn might lead to better treatment for other patients.

RISKS

Stem cell Choosing

We hope that stem cell chosing will mean that only normal blood making cells are returned to you/your child and we hope the growth factor treatment will make them grow faster. The major risk is that only a few stem cells are selected and the marrow will be slower than normal to recover. This might increase the risk of infection and bleeding. It is possible that the marrow would not recover at all. In that case we will give back the portion of your/your child's marrow that was frozen without treatment.

Marking

No bad effects have been seen in animal studies using these markers or in more than 30 people treated with marker genes here or at other institutions. Still, there may be risks. It is possible that the mouse virus might "recover" in the cell and be able to grow; it might even cause cancer. We think this is very unlikely but we cannot rule it out yet.

BONE MARROW TRANSPLANT

You (your child) will rest for one day after the chemotherapy. Then the bone marrow that was taken out before will be put back through an IV catheter. So that we can study the marked cells, we will take a little extra blood after the marrow is put back. The extra blood taken will be about 20 mL (1 tablespoon) once or twice a week for 6 weeks. We will also take extra blood monthly for 6 months, every six months for 2 years, and then once a year for 2 years.

LONG-TERM FOLLOW UP

We will follow your (your child's) progress over a long period. You (your child) will need to be examined yearly after the transplant. After the first 4 years this can be done by your (your child's) local doctor. A teaspoon of blood will be taken at each exam.

SIDE EFFECTS

Stem Cell Selection

May delay or prevent the graft from taking. May increase the risk of infection or bleeding.

Virus - No known side effects.

(Possibly could cause a few antibiotics such as neomycin or amikacin not to work or the virus could "recover" and grow or could make cells cancerous)

ALTERNATIVES

If you do not wish to have stem cell selection/marking treatment you/your child can still have high-dose chemotherapy and bone marrow transplant with untreated marrow.

STATEMENT OF UNDERSTANDING

The benefits of this experimental study are difficult to predict, and it is possible that no useful information will be gained. Participation in the study is strictly voluntary. If you/your child does not wish to volunteer, other options will be discussed and made available. Should any problems arise, one of the doctors will make you/your child aware of these developments and discuss plans of action.

1. I have read the material above and I willingly agree to take part/let my child take part in this study.

2. I have been able to talk as much as I want to with Dr. _____, who is taking care of me or my child _____, about the reasons for this study and about its risks.

3. I have been told about the other choices for treatment or for no treatment.

4. I know that this study may have risks that the doctors do not know about now.

5. I know that I/my child can withdraw from this study at any time. If this happens, I/my child can still be treated at St. Jude.

6. I know that I/my child will not be paid for being in this study.

7. I know that if I am injured/my child is injured because of this study, I/my child will not get any money for being injured. However, St. Jude will treat the injury, free of charge.

8. I know that my/my child's records will not be given to anyone outside the hospital unless I agree. I agree that my/my child's records may be reviewed by agencies such as the Food and Drug Administration or the National Cancer Institute.

9. I know that if I have more questions about this study or about any injury from the treatment, I can call _____ at _____.

10. I know that I can get more information about my/my child's rights as a study participant by calling the Chairman of the Institutional Review Board at 901/522-0300.

11. I will receive a signed copy of this statement.

_____ _____

Patient Parent

Physician	Witness
Date	Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

ASSENT FORM FOR PATIENTS AGED 7-18
USE OF DOUBLE MARKING WITH RETROVIRAL VECTORS TO DETERMINE RATE OF RECONSTITUTION OF UNTREATED AND CYTOKINE EXPANDED CD34+ SELECTED MARROW CELLS IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION

You are going to have an autologous bone marrow transplant as part of the treatment for your cancer. You will get strong chemotherapy to kill any cancer cells that may be left. Then, a bone marrow transplant will be done using your own bone marrow. This is necessary because the chemotherapy also kills normal cells in the marrow.

TREATMENT OF MARROW BY SELECTING STEM CELLS AND USING GROWTH FACTORS WITH GENE MARKING

After autologous bone marrow transplant there is a risk of infection and bleeding while the blood counts are low before the new marrow grows back. In this study we would like to find out if we can make the marrow grow faster after we put it back by mixing it with medicines which make the marrow grow. To do this, we will separate cells called stem cells which grow up to be normal marrow cells. We will split the stem cells into two parts and put a different marker gene into each of them. One marked part will then frozen without more treatment, and the second will be mixed with growth medicine to try and make the marrow come back faster. Both parts of the marrow will then be given back to you. We will then look to see if the treated part of the marrow grows back faster and stronger than the untreated part.

MARROW HARVEST AND MARKING

Before the high-dose chemotherapy starts, we will take out a small part of your bone marrow from the hip bone. This will be done in the operating room while you are asleep under anesthesia. You will not feel anything when the marrow is taken. There may be some pain later. You will be given medicine for the pain.

We will freeze about one-third of the marrow immediately with no treatment. This bone marrow will be saved as "back up" bone marrow to be given to you just in case the first bone marrow does not take. Stem cells will be picked from the rest of the marrow and half will be marked with LNL6 and half with G1N. These are the names of special mouse viruses that have been changed to keep them from causing infection. One half of the marrow will then be frozen immediately and the other will be mixed with growth medicines for 5-7 days and then frozen. Both parts will then be put back through your central line after the chemotherapy is finished. The markers let us follow the treated marrow in your body to see if the medicines makes the marrow grow back faster.

BENEFITS AND RISKS

Stem cell Selection

We hope that stem cell picking will mean that only normal blood making cells are returned to you and we hope the treatment will make them grow faster. The major risk is that only a few stem cells are selected and the marrow will be slower. This might increase the risk of infection

and bleeding. It is possible that the marrow would not recover at all. In that case we will give back the portion of your marrow that was frozen without treatment.

Marking
No bad effects have been seen with the marking in anyone. Still, there may be risks. It is possible that it "recover" in the cell and be able to grow; it might even cause cancer. We think this is very unlikely but we cannot rule it out yet.

ALTERNATIVES
If you do not wish to have stem cell selection/marking treatment you can still have high-dose chemotherapy and bone marrow transplant with untreated marrow.

STATEMENT OF UNDERSTANDING

You have the right to ask any questions you want and they will be answered. Before you have signed this form you have talked about the transplant with your doctor and all your questions have been answered

_____	_____
Patient	Parent
_____	_____
Physician	Witness
_____	_____
Date	Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

BIOGRAPHICAL SKETCH CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
HESLOP, Helen Elisabeth	Asst. Member, BMT; Hematology-Oncology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
U. Otago, Dunedin, New Zealand	MB ChB	1980	Medicine
Royal Australasian Col. of Physicians	FRACP	1987	Medicine
Royal Australasian Col. of Pathologists	FRCPA	1987	Hemat/Immunol
U. Otago, New Zealand	MD	1990	Hematology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1980-82 House Officer/Senior House Officer, Canterbury Hospital Board, Christchurch, New Zealand

1982-84 Medical Registrar, Canterbury Hospital Board, Christchurch, New Zealand

1984-85 Senior Registrar in Hematology, Canterbury Hospital Board, Christchurch, New Zealand

1986-89 Research Fellow/Honorary Lecturer, Royal Free Hospital, London, England

1989-91 Postdoctoral Research Associate, Department of Biochemistry and Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN

1991 to present Assistant Professor, Department of Pediatrics, University of Tennessee, Memphis, College Medicine, Tennessee

1991 to present Assistant Member, Disivison of Bone Marrow Transplantation, Department of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee

PUBLICATIONS (From a total of 57)

Heslop HE, Gottlieb DJ, Bianchi ACM, Meager A, Prentice HG, Mehta AB, Hoffbrand AV, Brenner MK. *In vivo* induction of gamma interferon and tumour necrosis factor by Interleukin 2 infusion following intensive chemotherapy or autologous marrow transplantation. Blood 74:1374-1380, 1989.

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Oblakowski P, Bello-Fernandez C, Reittie JE, Heslop HE, Galatowicz S, Veys P, Wilkes S, Hazlehurst G, Hoffbrand AV, Brenner MK. Possible mechanism of selective killing of myeloid leukemic blast cells by lymphokine-activated killer cells. Blood 77:1996-2001, 1991.

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Rencher SD, Hanif I, Heslop HE, Turner V, Brenner MK, Hurwitz JL. Reconstitution of the T-cell receptor $\alpha\beta$ repertoire in recipients of allogeneic bone marrow transplant. Bone Marrow Transplant 10:521-527, 1992.

Krance RA, Heslop HE, Mahmoud H, Ribeiro RC, Douglass EC, Hurwitz CA, Santana VM, Kun L, Horowitz MM, Brenner MK. Anti-pan T-lymphocytic ricin A chain immunotoxin (H65-RTA) and methylprednisolone for acute GVHD prophylaxis following allogeneic BMT from HLA-DR identical sibling donors. Bone Marrow Transplant 11:33-36, 1993.

Williams LL, Rooney CM, Conley ME, Brenner MK, Krance RA, Heslop HE. Correction of Duncan's syndrome (X-linked lymphoproliferative disorder) by allogeneic bone marrow transplantation. Lancet 342:587-588, 1993.

Brenner MK, Rill DR, Holladay MS, Heslop HE, Moen RC, Busche M, Krance RA, Anderson WF, Ihle JN. Gene marking to determine whether autologous marrow infusion restores long-term haempoiesis in cancer patient. Lancet 342:1134-1137, 1993.

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Koehler M, Hurwitz CA, Krance RA, Coustan-Smith E, Williams LL, Santana VM, Ribeiro RC, Brenner MK, Heslop HE. Xomazyme CD5 immunotoxin in conjunction with partial T cell depletion as GVHD prophylaxis in pediatric patients receiving matched unrelated donor bone marrow transplantation. Bone Marrow Transplant (in press).

Heslop HE, Brenner MK, Rooney CM, Krance RA, Roberts WM, Rochester R, Smith CA, Turner V, Sixbey J, Moen R, Boyett JM. Administration of neomycin resistance gene marked EBV specific cytotoxic T lymphocytes to recipients of mismatched related or phenotypically similar unrelated donor marrow grafts. Human Gene Ther 5:381-197, 1994.

CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
BRENNER, Malcolm Keith	Member, Hematology/Oncology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Membership, Royal College of Physicians	MRCP	1977	Medicine
University of Cambridge	PhD	1981	Immunology
Membership, Royal College of Pathologists	MRCPath	1988	Haematology
Fellowship, Royal College of Physicians	FRCP	1990	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1975 House Physician to Professor M.D. Milne, Westminster Hospital, London, U.K.

1976 Senior House Officer to Professor J.P. Shillingford, Hammersmith Hospital, and Royal Postgraduate Medical School, London

1977 Senior House Officer to Professor M. Turner Warwick and Dr. P. Cole, Brompton Hospital, London

1977-78 Medical Registrar to Professor A.F. Lant and Dr. L.W. Loughridge, Westminster Hospital, London, U.K.

1978-81 Medical Research Council Training (MRC) Fellow, Division of Immunology, Department of Pathology, Cambridge University, U.K.

1981-83 MRC Clinical Scientist, Honorary Senior Registrar, Northwick Park Hospital, and Clinical Research Centre, London

1983-85 Lecturer in Haematology, Royal Free Hospital and Hospital for Sick Children, Great Ormond Street, London

1985-89 Senior Lecturer, Honorary Consultant Haematologist (Deputy Director, BMT Program) Royal Free Hospital, London, U.K.

1990 to present Professor, Departments of Pediatrics and Medicine, University of Tennessee, Memphis, Tennessee

1990 to present Member, Hematology/Oncology, and Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee

SELECTED PUBLICATIONS (of 137 total)

Heslop HE, Gottlieb DJ, Bianchi ACM, Meager A, Prentice HG, Mehta AB, Hoffbrand AV, Brenner MK. *In vivo* induction of gamma interferon and tumor necrosis factor by interleukin-2 infusion following intensive chemotherapy or autologous marrow transplantation. Blood 74:1374-1380, 1989.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
KRANCE, Robert Allan	Member, Hematology-Oncology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Loyola University, Chicago, IL	B.S.	1966	Biology
Loyola University, Chicago, IL	M.D.	1970	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1971-72 Resident in Pediatrics, Rush Presbyterian St. Luke Medical Center, Chicago, Illinois
1972-74 United States Air Force, Dyess Hospital, Pediatric Clinic, Abilene, Texas
1974-75 Resident in Pediatrics, St. Jude Children's Research Hospital, Memphis, Tennessee
1975-77 Fellow, Pediatric Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN
1977-77 Research Associate, St. Jude Children's Research Hospital, Memphis, Tennessee
1977-79 Staff Hematologist-Oncologist, Children's Hospital at Stanford, Palo Alto, California
1978-79 Clinical Assistant Professor of Pediatrics, Stanford University School of Medicine, Stanford, CA
1979-87 Assistant Member, Division of Pediatrics, City of Hope National Medical Center, Duarte, CA
1982-87 Clinical Assistant Professor of Pediatrics, University of Southern California at Los Angeles County Medical Center, Los Angeles, California
1984-87 Principal Investigator for Pediatric Oncology Group at City of Hope National Medical Center, Duarte, California
1985-87 Chief, Pediatric Bone Marrow Transplantation, City of Hope National Medical Center, Duarte, California
1987-87 Associate Member, City of Hope National Medical Center, Duarte, California
1987-90 Director, Clinical Bone Marrow Transplantation, Children's Hospital Hospital of Orange County, Orange, California
1990-94 Associate Member, Department of Hematology-Oncology and Clinical Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee
1994 to Member, Department of Hematology-Oncology and Clinical Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee

PUBLICATIONS

Lauer SJ, Pinkel D, Buchanan GR, Sartain P. Cornet JM, Krance RA, Borella LD, Casper JT, Kun LE, Hoffman RG, Camitta BM. Cytosine arabinoside/cyclo-phosphamide pulses during continuation therapy for childhood acute lymphoblastic leukemia. Potential selective effect in T-cell leukemia. Cancer 60:2366, 1987.

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CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

9406-077 Deisseroth, Albert; Hortobagyi, Gabriel; Champlin, Richard; and Holmes, Frankie; MD Anderson Cancer Center, Houston, Texas; *Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Breast Cancer: A Pilot Trial.*

Date of RAC Approval: 6-9-94
Date of NIH Approval: 7-12-94

PART III-C

SCIENTIFIC ABSTRACT

Replication incompetent, recombination incompetent retroviral vectors will be used to introduce chemotherapy resistance (MDR-1) cDNAs into the normal stem cells of autologous peripheral blood removed and stored following chemotherapy delivered to patients with breast cancer who are poor risk, and therefore at very high risk (80%) of dying of their disease. We estimate that between 0.6 and 2 x 10^6 CD34 positive cells/kg will be infused and that among these, 50% of the total CD34 selected cells infused will be exposed to a MDR-1 containing vector. We will look for the number of MDR-1 marked cells using a methylcellulose late progenitor colony culture system and a PCR assay for the MDR-1 gene. In addition, we will monitor the acquisition of chemotherapy resistance by stem cells of varying degrees of immaturity by using culture assays for these cells under chemotherapy selection (methylcellulose assay and colonies grown from Dexter cultures incubated for more than 35 days using PCR for MDR-1. These studies will help us evaluate if introduction of MDR-1 cDNA into peripheral blood will confer chemotherapy resistance on these cells, thus allowing therapy of a greater level of intensity of taxol to be delivered following transplant and therefore change the course of poor prognosis breast cancer in these patients.

NON-TECHNICAL ABSTRACT

Autologous transplantation is a technique which makes safe the very high doses of chemotherapy which are required to eradicate some populations of breast cancer cells. Although the responses seen in poor prognosis breast cancer patients is often dramatic following the intensive therapy used in autologous transplantation, most patients who respond do not exhibit increased survival. This indicates that additional therapy following recovery from the transplant might be beneficial. In order to make possible the delivery of relatively intensive doses of taxol following autologous transplant, we will introduce the MDR-1 cDNA into the stem cells of patients with breast cancer before storage for transplant. The peripheral blood stem cells for transduction is removed from the patient at the time of remission induced by conventional dose chemotherapy, stored and re-infused into the patient after intensive therapy in order to restore marrow function. To accomplish this, 50% of the peripheral blood cells stored from breast cancer patients will be incubated with the multidrug resistance (pVMDR-1) chemotherapy resistance vector. This vector will introduce a MDR-1 cDNA into the peripheral blood cells. The establishment of this gene in the cells will promote their growth over unmodified cells following taxol chemotherapy given following the autograft. If this is the case, we will be in a position to safely deliver high dose taxol after autologous transplant in breast cancer patients and thereby alter the unfavorable natural history of this disease. The results of this study will be used to improve the therapy given to patients with breast cancer. It is not designed to benefit the patients themselves.

THE UNIVERSITY OF TEXAS
M.D. ANDERSON CANCER CENTER
Division of Medicine

Protocol Title: Use of safety-modified retroviruses to introduce chemotherapy resistance sequences into normal hematopoietic cells for chemoprotection during the therapy of breast cancer: A Pilot Trial

1.0 OBJECTIVES

2.0 BACKGROUND

3.0 BACKGROUND DRUG INFORMATION

4.0 PATIENT ELIGIBILITY

5.0 TREATMENT PLAN

6.0 PRE-TREATMENT EVALUATION

7.0 EVALUATION DURING STUDY

8.0 CRITERIA FOR RESPONSE AND TOXICITY

9.0 CRITERIA FOR REMOVAL FROM STUDY

10.0 STATISTICAL CONSIDERATIONS

REFERENCES

APPENDIX A1: Zubrod Performance Scale
APPENDIX A2: Cardiac Status Scale
APPENDIX B: Ceprate Stem Cell Collector
APPENDIX C: Manuscripts on MDR-1 Transduction
APPENDIX D: Preparation and Conditions of Exposure of Retrovirus
APPENDIX E: NCI Common Toxicity Criteria
APPENDIX F: Evaluation Before and During Therapy
APPENDIX G: Immunohistochemistry for breast cancer
APPENDIX H: Background Drug Information
APPENDIX I: Statistical Analysis of Trial Results
CONSENT FORM

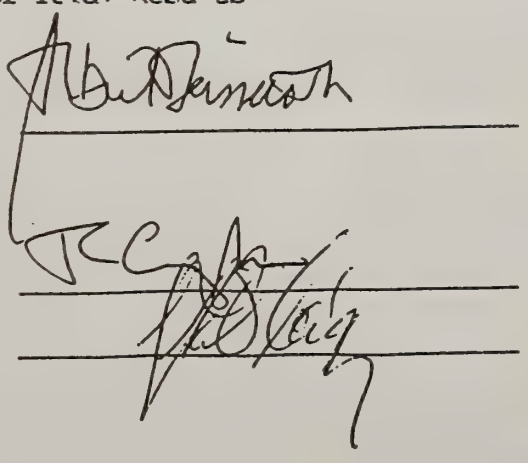
Study Chairman:

Albert B. Deisseroth, M.D., Ph.D.

Study Co-Chairman:

Richard Champlin, M.D.

Gabriel Hortobagyi, M.D.



Study Co-Investigators:

John Kavanaugh, M.D.

Martin Korbling, M.D.

Jeane Hester, M.D.

David Claxton, M.D.

Borje Andersson, M.D., Ph.D.

Donna Przepiorka, M.D., Ph.D.

Koen van Besien, M.D.

Rakesh Mehra, M.D.

Elie Hanania, Ph.D.

Richard Giles, Ph.D.

Siqing Fu, M.D.

Michael Andreeff, M.D., Ph.D.

Debra Ellerson

Leslie Calvert

Joy Hamer, RN

U. T. M. D. Anderson Cancer Center, Departments of Hematology and Medical Oncology, 1515 Holcombe Blvd., Houston, TX 77030; (713) 792-8750.

PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title) (Two lines not to exceed 75 characters per line)

Introduction of Chemotherapy Resistance into Normal Marrow Cells

STUDY CHAIRMAN: Albert Deisseroth, M.D., Ph.D., Gabriel Hortobagyi, M.D., and Richard Champlin, M.D.

Patient Eligibility: (Twenty lines not to exceed 75 characters per line)

1. Patients with Stage III/IV breast cancer who have failed initial therapy or reinduction therapy, and who have not been previously exposed to Taxol, and who have measurable disease, and who have not had irradiation to greater than 20% of marrow bearing areas. Patients must be at least partially responsive to chemotherapy.
2. Age 18 to 60 years
3. Performance status 0-2 and acceptable cardiac, renal, hepatic, and pulmonary functions.
4. Breast cancer patients must have a negative bone scan and immunohistochemmical staining tests (Appendix G) which are negative for breast cancer cells.

Treatment Plan: (Include dose adjustment) (Twenty lines not to exceed 75 characters per line)

1. Breast cancer patients with disease which is only partially responsive to conventional dose chemotherapy will have peripheral blood stem cells harvested with GCSF stimulation, following administration of non ablative dose of cyclophosphamide (4 gms/m^2). Autologous peripheral blood stem cells will be subjected to concentration in the COBE machine concentrator and CD34 selection in the CellPro Stem Cell Concentrator. One half of these CD34 selected cells will then be modified with a safety-modified retrovirus which carries a cDNA for the multidrug resistance MRNA (MDR-1).
2. Infusion of the modified and unmodified peripheral blood stem cells will take place on d 0, following delivery of high dose cytoxan (1.5 g/m^2 on d -8-,-7,-6), thiotepa (200 mg/m^2 d-8,-7,-6) and BCNU (150 mg/m^2 d-8,-7,-6). Mesna will be given during the cyclophosphamide infusion. GCSF will be administered post transplant. Benadryl 25 mg and solucortef 100 mg will be given before bone marrow infusion to prevent anaphylaxis.
3. Patients will receive prophylactic oral antibiotics and blood products irradiated with 2500 rads.
4. Following recovery from transplant, Taxol will be given starting at 60 mg/m^2 CI qdx1, and then be given at increasing doses each course (see Section 5.4) to tolerance given every three weeks, for a total of 12 courses.

Patient Evaluation:

(Twenty lines not to exceed 75 characters per line)

	Pre-Treat.	Every 2-3 Days	When WBC Count > 1.5 K/μl Every 3 Months in Remission
History, Phys. Exam	X		
CBC, differential and platelet counts	X	X	X
SMA12/PT/PTT/FIB/FSP and electrolytes*	X	X	X
BM aspirate & BX**	X		X
BM cytogenetics	X		X
EKG/CXR/urinalysis*	X		
Pulmonary function test	X		
FDA QC Tests as outlined in post therapy evaluation section.	X		X

* In addition as indicated by clinical and hematologic situations.

** Bone marrow aspirate and peripheral blood for PCR and functional evaluation of the MDR content of the cells at +21, +32 days post marrow reinfusion, and every three weeks during the first 6 months every 3 months during the first 2 years, and every 6 months for the first 5 years following transplant.

The peripheral blood and bone marrow will be studied with immunohistochemical staining for the presence of breast cancer cells, to determine if the patient is eligible for the MDR-1 transduction (see Appendix G).

Miscellaneous Information: (Include any other information that you feel is pertinent to the study)

(Three lines not to exceed 75 characters per line)

None

Statistical Considerations:

(Twelve lines not to exceed 75 characters per line

We plan to initially enter 15-20 advanced breast cancer patients in this pilot trial so that 10 patients are fully evaluable on the trial. We estimate that 100% of the patients will exhibit evidence of the functional viral MDR-1 sequences since chemotherapy selection in vivo will be used to select for the hematopoietic cells which have retained the MDR-1 retroviral transgenome.

Objectives:

(Twelve lines not to exceed 75 characters per line

1. To evaluate the feasibility of introducing into early hematopoietic progenitor cells the MDR-1 cDNA in advanced breast cancer patients and thereby promoting the in vivo selection of genetically-modified hematopoietic cells which are resistant to chemotherapy by virtue of having been transduced by the MDR-1 cDNA containing safety-modified retroviruses.

2. To determine the toxicity of modifying stem cells with MDR viruses on hematopoietic function following and during transplant.

OBJECTIVES

1.1 To test the feasibility of introducing the MDR-1 cDNA into normal hematopoietic early progenitor cells in advanced breast cancer patients through transduction of these cells with a safety-modified retrovirus which bears the MDR-1 cDNA in a transcription unit.

1.2 To determine the toxicity of modifying normal stem cells with MDR viruses on hematopoietic function following and during transplant.

BACKGROUND

Breast cancer strikes women in the prime of life. For those patients whose disease does not respond to regional approaches or conventional dose chemotherapeutic approaches to the control of the disease, an 80% mortality can be predicted.

The poor response of advanced breast cancer led to the testing of the efficacy of intensive dose combination chemotherapy (1). Methods of separating normal cells and breast cancer cells and detecting the presence of 1 cancer cell in 10,000 normal cells (see Appendix G). The early trials with intensive therapy and autologous bone marrow transplantation of breast cancer did not lead to measurable increases in survival, although dramatic responses in terms of reductions in bulk disease were often seen (1-2). This suggested that the delivery of intensive but not ablative cyclical therapy following autologous bone marrow transplantation would lead to an improvement of survival. Unfortunately for these patients, the marrow graft is very fragile following transplantation such that it is not always feasible to deliver relatively intensive cyclical therapy following bone marrow transplantation.

During the early 70's, many investigators identified that epithelial neoplasms exhibited resistance to multiple drugs, and that if the cells were resistant to one member of this family of drugs, the tumor cells were often resistant to the other members of the family. Work designed to isolate this efflux pump protein culminated in the isolation of the p170 glycoprotein and the MDR-1 cDNA and gene.

Subsequent laboratory work on tumor cell lines which exhibited resistance to these drugs, or on tumor cell lines into which the cDNA for the MDR- 1 cDNA was introduced by transduction, led to the conclusion that the MDR-1 cDNA conferred resistance to the following drugs: actinomycin D, VP-16, Velban, vincristine, adriamycin, and Taxol. In addition, exposure of the cells of patients leads to increases in the levels of the MDR-1 mRNA and in the protein GP170 for which it is the coding sequence.

Studies of patients with breast cancer have shown that the elevation or even the presence of detectable levels of the MDR-1 mRNA or protein in breast cancer cells is very uncommon (3-5), and that even after exposure to the MDR-1 drugs, that the levels of elevated MDR-1 (which are 2 to 3-fold higher than those which are detectable before treatment), are at least 3-fold lower than the levels which are achievable in hematopoietic stem cells which have been transfected with

safety-modified retroviruses which carry a single MDR-1 cDNA in a transcription unit (6). In addition, animal model experiments in dogs, mice and in primates, have shown that it is feasible to modify the hematopoietic stem cells with safety-modified retroviruses which carry the MDR-1 cDNA in man or the MDR-3 cDNA in the mouse such that the hematopoietic cells following bone marrow transplant retain significant levels of cells which are genetically modified, and express the MDR-1 cDNA (5,7-8). Furthermore, these studies have shown that delivery of conventional dose chemotherapy to these animals results in increases in the levels of the MDR-1 cDNA and GP170 protein in the marrow and peripheral blood cells of these animals, and in the percentage of cells which carry the retroviral transgenome. In other words, these studies have shown that it is possible to increase the resistance of hematopoietic cells to the effects of MDR-1 drugs and that this increased resistance, which the MDR- 1 retrovirus transduction confers upon the hematopoietic stem cells, results in the ability to select and maintain the genetically modified cells in the marrow of animals which have been transplanted with these modified cells by autologous bone marrow transplantation. No alteration in the maturation of hematopoietic cells has been seen in mice transplanted with MDR modified cells (8,14). Several groups have now shown that the presence of viral MDR-1 in transplanted marrow is resistant to Taxol and other MDR drugs (11-14).

We have proposed the use of peripheral blood CD34 positive cells for the transplant. Our institution has had experience with transplantation with the CD34 selected cells. We have seen recovery of the absolute neutrophil count to 500/cu mm in 10, 14, 14, and 17 days in breast cancer patients who have been infused with peripheral blood cells and 14, 17, 18, 18, 24, and 27 days in advanced CML patients who have been transplanted with CD34 selected peripheral blood and marrow cells. The University of Denver has observed an average of 17 days to 500 neutrophils/cu mm in advanced breast cancer patients.

As shown in Figure 1A, our own laboratory (see Appendix C) has shown that introduction of the MDR-1 cDNA containing safety-modified retrovirus into the marrow cells of Balb-C mice before transplant shows that the marrow cells of the mice transplanted with the MDR-1 modified marrow are more resistant to Taxol than are mice transplanted with unmodified marrow. In addition, as shown in Figure 1B, sequential transplantation of these MDR-1 modified cells generates hematopoietic recovery which is Taxol-resistant through three successive transplants (see Appendix C). This shows that early progenitor cells can be modified by our MDR-1 procedure. In addition, in vitro data with CD34 selected progenitor cells shows that the long-term culture initiating cell is transduced by this virus, that the transduction frequency is 10%, and that the levels of MDR-1 mRNA from the viral MDR-1 are much higher than the endogenous MDR-1, and that this translates into Taxol resistance.

The marrow becomes resistant to Taxol chemotherapy using transduction procedures which give integration of the virus into only 1-10% of the cells suggesting that in vivo selection of the MDR-1 modified cells occurs during chemotherapy. During the past two years, considerable progress has been made in the introduction of additional sequences into the somatic cells of patients in both constitutional as well as acquired molecular diseases (9-13). This has been accomplished with a

family of safety-modified retroviruses which have been modified so that they cannot replicate in the normal or abnormal somatic cells of the patients. The sequences required for viral replication have been removed from the viruses, and placed in fragments in different parts of the genome of "producer cell lines", in which the replication incompetent safety-modified viruses can occur. These viruses are not harmful so long as they are not contaminated with replication competent helper virus, or viruses which can combine with the safety-modified viruses in a way which preserves the functional MDR- 1 transgenome and restores (integrates) the rest of the replicating functions into the virus in an active array.

Producer cell lines can support the proliferation and replication of safety-modified viruses. The producer cell lines contain the replicative function of the virus. The safety-modified viruses can be grown in these cell lines. The use of safety-modified viruses from the cell lines has not led to the appearance of replication competent virus over the past two years in 250 laboratories. In addition, the use of these retroviruses in patients has not led to any abnormal disease state, or toxicity in patients in whom these viruses have been used for therapy (15). This data led to several therapeutic trials which involved the modification of the somatic cells of patients for genetic marking and for the replacement of missing or defective genes in a number of diseases. We have been involved in this work at M.D. Anderson, and have at the moment four protocols approved by the NIH Recombinant DNA Advisory Committee, and two protocols FDA-approved and ongoing at the M.D. Anderson Cancer Center at this time (16-19). We have shown in our own laboratory that we can achieve transduction frequencies in the 1-5% range even with the normal hematopoietic progenitor cells (17). Malcolm Brenner of St. Jude Children' s Research Hospital has shown that these vectors, when used to modify the marrow of AML patients in remission, lead to a hematopoietic population following transplant which is positive for the retroviral transgenome at a frequency after transplant which equaled the frequency of the marrow cells following in vitro exposure to the MDR viruses before transplant. We have similar data in CML patients, a report of which is in press in Blood (see Appendix C). These studies have also shown that the retroviruses is functional by the criterion of resistance to the bacterial resistance gene Neo. The presence of the retroviral transgenome in the cells has persisted up to greater than 1-1/2 years.

Figure 1A

Myelosuppression in Control Mice and MDR Transduced Mice After Taxol Chemotherapy - 20 mg/kg

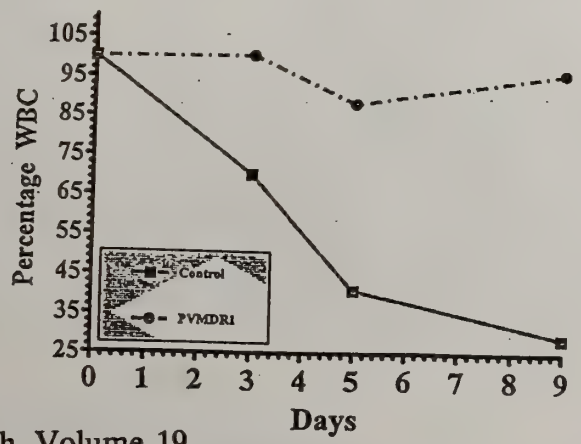
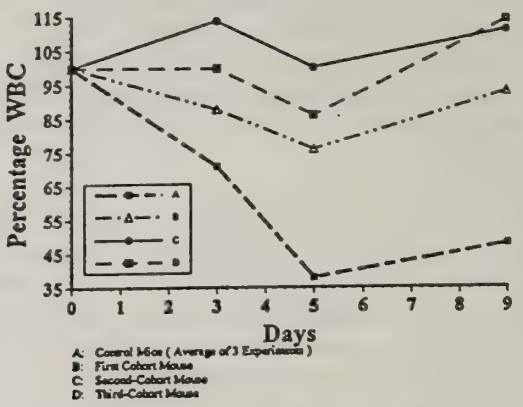


Figure 1B



Interestingly, the studies of Brenner have been positive, the AML cells of these patients are also positive at the 10% level at the time of relapse, suggesting that all of the cells of marrow exposed to the safety-modified retroviruses are modified at the same level with the vector sequences. The data and our animal model data suggests that we will be able to modify the normal hematopoietic stem cells of these patients with the retroviruses which are safety-modified and contain a functional transcription unit with MDR-1 cDNA.

Accordingly, we have proposed to modify the peripheral blood stem cells of breast cancer patients, who are incompletely responsive to conventional dose chemotherapy, and whose expectation of dying from the disease (by virtue of adverse prognostic features) is 80% or more. In this program, we will harvest the peripheral blood stem cells, following non ablative dose of cyclophosphamide chemotherapy (4 gms/^{2}), purify the early progenitor cells through CellPro CD34 selection, and then modify the peripheral blood cells with a protocol outlined in Appendix D. GCSF, 5 mcg/kg, will be given as a daily subcutaneous dose starting with the completion of the non ablative dose chemotherapy. The purpose of this chemotherapy and GCSF is first to produce the optimal conditions for the collection of early progenitors from the peripheral blood. This is a regimen with which The U.T. M.D. Anderson has had extensive experience in producing preparations which are very rich in reconstituting cells, if the collections are started when the total white cell count is between 800/cu mm and 2,000/cu mm. A second purpose of this regimen is to produce conditions under which the cells which are collected from the peripheral blood contains higher numbers of early hematopoietic precursor cells which are proliferating. This is necessary to generate conditions under which the early precursor cells will be modified by the MDR-1 virus, as the conditions of a recovering marrow produce that condition. The peripheral blood cells collected are split into two halves, and one half is exposed to the virus after CD34 selection. The CD34 selection is used to increase the frequency of cells with long term reconstituting capability so as to increase their frequency of being modified by the virus. The protocol of non

ablative dose of cyclophosphamide chemotherapy (4 gms/m^2), collection of CD34 selected peripheral blood cells early in the course of hematopoietic recovery (the precise time of which may vary for each patient but is usually as soon after a total white cell count of 2,000/cu mm has been reached), has produced transduction frequencies of the very early precursor cells, the long-term marrow culture initiating cells (LTCIC) in the range of between 5-20% in our own work and in that of others. The peripheral blood cells will be CD34 selected, and then incubated in vitro in the retroviral supernatant to introduce the viral MDR-1 into the patient's hematopoietic stem cells. We will then treat the systemic disease a single time before transplant, with a regimen which consists of high dose cyclophosphamide (1.5 g/m^2/d x 3) thiotepa (200 mg/m^2/d x 3), and BCNU (150 mg/m^2 qd X 3). This regimen was chosen as it has agents which are active in breast cancer, and at the same time will provide suppression of the endogenous hematopoiesis needed to facilitate engraftment of the genetically-modified cells. We have significant institutional experience with this protocol. Following this single course of intensive systemic therapy, these patients will be transplanted with the genetically modified stem cells. Following recovery of hematopoietic function, the patients will receive cyclical Taxol therapy with starting dose at 60 mg/m^2. The dose will be increased by a dose adjustment schema described in Section 5.4 from 60, 120, 180, 225, and 275 mg/m^2. This will enable us to increase the dose as the percentage of transduced cells increases without imposing toxicity on individual patients. The courses will be delivered every three to four weeks to a total of 12 courses, depending on recovering the ANC to 2000/mm^3 and the platelet amount to 100,000/mm^3. We will measure the levels of the cells which are modified by the MDR-1 viruses, and monitor the rate of hematopoietic recovery, which should become more rapid with each cycle and should contain higher and higher percentages of MDR-1 cDNA positive cells due to the selective effect of the chemotherapy. Finally, once this principle of in vivo selection is established, it will be possible to increase the doses of Taxol with each cycle of therapy. The goal of this program is to establish this principle of in vivo selection of genetically modified cells, and to study their effect on the ability to deliver very intensive doses of combination chemotherapy, or at least in the initial phases, relatively intensive doses of Taxol following recovery of hematopoietic function after autologous stem cell marrow transplantation. This may have a beneficial effect on the survival of these patients in whom the inability to eradicate residual disease lead to an 80% expectation of dying of their disease within two years.

3.0 DRUG BACKGROUND INFORMATION (See Appendix H)

4.0 PATIENT ELIGIBILITY

4.1 Advanced disease Stage III/IV breast patients who have not previously been exposed to Taxol and who have failed conventional dose therapy or induction therapy, who therefore have an 80% chance of dying of progressive cancer. Patients must be however at least partially sensitive to conventional dose chemotherapy. Patients must have measurable or evaluable disease and have not had irradiation to greater than 20% of marrow bearing areas.

Patients who have had congestive heart failure or coronary artery disease or who have an ejection fraction of less than 50% (by Cardiac Muga Scan) will not be eligible for this protocol.

4.2 Patients must have a performance of <2 on Zubrod performance scale (Appendix A1), a creatinine level <1.6 mg%, normal creatinine clearance, acceptable cardiac condition (class I or II; Appendix A2), normal liver functions (the SGOT should be within the normal range) with bilirubin <2 mg%, and acceptable pulmonary function tests (FEVI and DLCO >50% of predicted). Patients should be free of infections at the time of treatment.

4.3 Patients who have a history of bowel obstruction or greater than Grade I neuropathies will be excluded. Patients should have had a recent marrow exam and have had no evidence of involvement with breast cancer. In addition, the peripheral blood aned marrow must be negative for breast cancer cells by the immunohistochemical assays summarized in Appendix G.

4.4 Patients with refractory disease must have an engrafting dose of a CD34 selected autologous peripheral blood stem cells stored one half of which have been CD34 selected, and then exposed to safety-modified retroviruses which contain the MDR-1 cDNA in a transcriptional unit. A dose of backup marrow or peripheral blood stem cell collection which is equivalent to an engrafting dose must be stored. Patients must sign informed consent, must not be pregnant or lactating, and must be practicing adequate contraception if of childbearing potential.

4.5 No patient who is unresponsive to platelet infusions will be eligible for this study unless a total of 8 collections of autologous platelets are available before therapy.

Before post transplantation Taxol therapy is initiated, the patients must have an ANC of 1800/cu mm, and a platelet count and hemoglobin equal to or greater than 100,000/cu mm and 8 g/dl, respectively. These levels must also be imposed for eligibility for the protocol.

5.0 TREATMENT PLAN

5.1 General: All patients should be registered with the data management office, Ext: 2-2926. Information pertaining to important patient characteristics will be recorded.

5.2 Bone marrow aspiration and collection of peripheral blood stem cells and storage: In these breast cancer patients, two engrafting doses of autologous peripheral blood or marrow cells (one engrafting dose of which is CD34 selected peripheral blood stem cells) will be collected in the early phases of hematopoietic recovery from conventional dose cyclophosphamide chemotherapy (4 gms/m^2) under GCSF stimulation (5 mcg/kg/day). The adequacy of a peripheral blood stem cell collection (2 engrafting doses) will be 4 x 10^8 nucleated cells/kg or 20,000 CFUGM/kg or 1 x 10^6/kg CD34 positive cells. These peripheral blood stem cells will be CD34 selected and by the CellPro Ceprate

Stem Cell Collector (see Appendix B) and one half of these cells (1/2 x 10^6/kg) will be exposed to safety-modified retroviruses which carry the MDR-1 cDNA in transcriptional unit as outlined in Appendix D. These cells must be negative for breast cancer cells by the immunostaining tests outlined in Appendix G. A backup marrow or peripheral blood stem cell collection which is equivalent to an engrafting dose will be made for breast cancer patients.

5.3 Treatment Plan: All patients should be treated in the Protected Environment if available.

a. The preparative ablative regimen, which is given a single time before the transplantation of MDR-1 modified and unmodified peripheral blood stem cells, will consist of the following systemic chemotherapy:

Cyclophosphamide: 1.5 g/m^2 qd on days -8,-7, and -6; for a total dose of 4.5 gms/m^2 over the three days, Mesna, 500 mg/m^2, will be given IV 1/2 hour before the first dose of cyclophosphamide, then 2 gms/m^2 will be given as a continuous infusion over each 24 hour period during the cyclophosphamide infusion.

Thiotepa at a dose of 200 mg/m^2 qd x 3 days, will be diluted in normal saline, and given IVPB over 4 hours on days -8,-7, and -6 at a total dose for the three days of 600 mg/m^2.

BCNU at a dose of 150 mg/m^2 will be dissolved in 100 cc of D5W and given dIV piggyback on days -8,-7, and -6 over 40 minutes (total dose 450 mg/m^2).

b. Subsequently, MDR-1 virally unmodified and modified autologous peripheral blood stem cells will be reinfused on day 0, which is 5 days after the last dose chemotherapy so as to protect the marrow from metabolites of the chemotherapy. Premedication with benadryl 25 mg and solucortef 100 mg ivb 30 minutes before reinfusion to prevent anaphylactic reactions.

c. Treatment of patients in the Protected Environment on the 12th floor of the Lutheran Pavilion is strongly urged. Patients will remain there until the attainment of 500 granulocytes/mm^3.

d. Patients will receive bactrim DS po BID and ketoconazole 200 mg po q8h as antibiotic prophylaxis or other antibiotics specified by the Infectious Disease group while hospitalized to protect patients against overwhelming infection.

e. All blood products will be irradiated from the start of treatment and for three months following the transplant.

f. GCSF (5 mcg/kg) will be given SC qd starting on day 1 after marrow harvesting and until the ANC is $\geq$10,000/μd.

5.4 Maintenance Taxol Therapy: Following recovery from the autologous transplant, 12 courses of Taxol will be given, starting at a dose of 60 mg/m^2, and escalating each course by the scheme outlined below (at 120, 180, 235, and 275 mg/m^2 depending on the grade of hematopoietic toxicity in the previous cycle of therapy) as a single 24-hour continuous infusion each cycle, in the doses listed below, every three to four weeks following recovery of hematopoietic function after transplantation of the MDR-1 virally modified peripheral blood stem cells, when the platelet count, hemoglobin, white cell count and ANC are greater or equal to the following values respectively: 100,000/cu mm, 8 g/dl, 4,000/cu mm, and 1800/cu mm. Each dose of Taxol will be preceded by Dexamethasone (20 mg po or IV at 12 hrs and 6 hrs prior to Taxol), Cimetidine (300 mg IV 30 min prior to Taxol), and diphenhydramine (50 mg IV 30 min prior to Taxol), to reduce the probability of analyphylaxis from Taxol. A total of 12 doses of Taxol will be given post transplant unless the events listed in Section 10.0 as stopping rules occur.

We have described below the algorithms used to adjust downwards the dose of Taxol if Grade IV toxicity is encountered following 60 mg/m^2 for the initial dose of Taxol.

We have proposed a starting dose of Taxol post autologous transplant of 60 mg/m^2. This dose is below the MTD, but was chosen because patients have decreased stem cell reserve immediately following transplant on the basis of decreased bone marrow cellularity. No one has given Taxol immediately following bone marrow transplant, and therefore we do not know if the MTD is the same for Taxol in these sensitive post transplant patients as it is in patients not just recovered from an autologous transplant. The discussions within our own center, and during the approval process before the NIH RAC led to the selection of the low starting dose of Taxol post transplant. As shown below, a dose escalation scheme which is rapid, and provides adjustments on the basis of the tolerance of the patient to the Taxol, is provided. This dose escalation scheme will provide for dose reductions in the case of grade IV toxicity, and dose escalations in case of the relative absence of toxicity (Grade I/II hematopoietic toxicity).

This low starting dose will not compromise the selection of genetically modified cells post transplant, since the dose escalation of the Taxol is so rapid. The studies in the mouse show that the frequency of the genetically-modified cells is quite high following bone marrow transplant, even before the administration of the first dose of Taxol (see Appendix C). This starting dose will not compromise the comparability of the recovery curves in patients transplanted with genetically-modified cells, with those breast cancer patients receiving comparable doses of Taxol, since the doses of Taxol which are comparable to those being administered to comparable breast cancer patients who have not had transplants with genetically-modified cells, will be reached through the dose escalation scheme very rapidly.

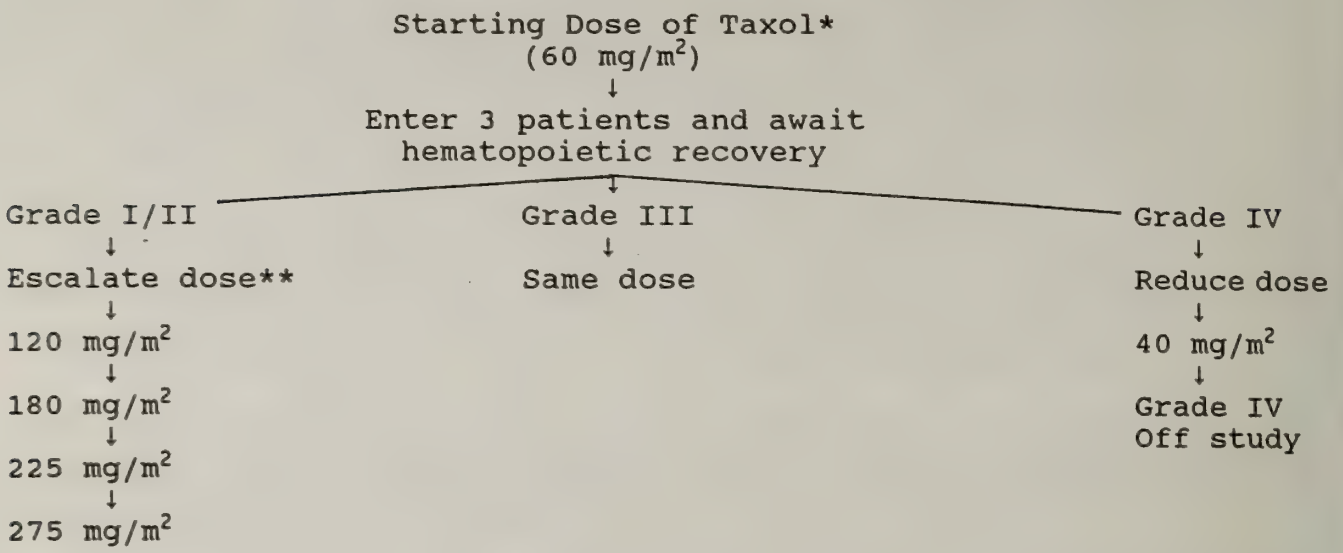
In addition, each patient will serve as her own control, since we will be comparing the depth and duration of the nadirs post taxol following each dose of Taxol, during the dose escalation scheme. In this process, we predict that the depth and duration of the nadirs following each dose of Taxol will become less marked as the dose of Taxol is increased in each succeeding cycle, since the percentage of genetically-modified cells will be increasing through selection. This is in contrast to the depth and duration of the nadirs following each dose of Taxol, in the patient in whom no genetically-modified cells are present, would be more and more severe with each succeeding cycle. In addition, we will be comparing the cells which are genetically-modified with the MDR-1 retroviral sequences, with the depth and duration of the nadir following each succeeding Taxol. This analysis should serve as a test of the performance of the pVMDR-1 retroviral transgene.

The Taxol will be administered as a 24-hour continuous infusion. GCSF will be given as a 5 mcg/ml subcutaneous injection daily following completion of the administration of Taxol chemotherapy. Since the patients who are not transplanted with MDR-1 modified cells, and who are receiving Taxol, are also receiving GCSF, in order to make our MDR transduction program of therapy comparable to the programs which are being given to advanced breast cancer patients who have not received genetically-modified cells, we have decided to administer GCSF. This has been approved by the NIH RAC in a previous protocol (RAC #9306-044), as well as by our own institutional IRB.

Before the beginning of the Taxol post transplant therapy, the patients must be totally recovered from the side effects of the transplant, they must have a performance status of 1 or less. The white cell count must be greater than 4,000/cu mm, the ANC must be 1800/cu mm or greater, and the platelet count must be equal to or greater than 100,000/cu mm. The patients must be afebrile, and have no evidence of ongoing infection before the initiation of the Taxol.

In the diagram below, we have summarized the algorithm to be used for dose adjustments for all subsequent Taxol courses in the case of Grade II or lower levels of toxicity following the first course of Taxol at 60 mg/m^2. This provides for a rapid escalation of the dose with each cycle, so that tumoricidal doses of therapy can be reached quickly. This algorithm also provides for the downward adjustment of the doses in the event of Grade IV toxicity. Grade III toxicity mandates a stable dose. Thus, this plan will combine restrictions on dose in the beginning, when the patients will be the most sensitive, and rapid escalation of the dose of Taxol, as fast as the evolution of the resistance to Taxol in the marrow of the patients post-transplant will allow.

DOSE ADJUSTMENT SCHEMA FOR PATIENTS POST-TRANSPLANT WITH MDR-1 MODIFIED MARROW



* For the initial dose only, if greater or equal to 2/3 of the first three patients have Grade IV toxicity, then start next cohort of three patients at 40 mg/m^2; otherwise, all patients will start at 60 mg/m^2.

** Rule for all subsequent courses after the first course for escalation or reduction:

Grade I/II toxicity leads to an increased dose level
Grade III same dose level
Grade IV reduce dose level

Patients will be followed for signs of a toxic effect of the transduction on the hematopoietic cells used to generate hematopoietic recovery following infusion of the modified marrow. Our data in transplants of eight CML and eight breast cancer patients reconstituted with the CD34 peripheral blood (breast) or peripheral blood and marrow (CML) hematopoietic cells suggests that the time required for recovery to 500 PMN/cu mm is in the range of 10-23 days. Any delay beyond 40 days, if it occurs in three successive patients, will be considered a sign that the retroviral modification has had a toxic effect on the autologous cells used for transplant.

The time to recovery after each dose of Taxol will be monitored by measuring the time required to reach 500 neutrophils/cu mm, 1000 neutrophils/cu mm, and the depth of the nadir will be measured after each cycle by Taxol. We will compare duration and depth of each nadir (time to recover to 500 and 1000 PMN/cu mm, and the depth of each nadir) encountered after the different doses of Taxol summarized above in breast cancer patients who have been transplanted with MDR-1 transduced marrow, with duration and depth of neutropenia encountered in breast cancer patients whose marrow is not MDR-1 transduced. This will tell us if the MDR-1 transduction is protecting the marrow.

RT PCR will be conducted to determine the percentage of cells which contain the MDR-1 retroviral vector to measure the frequency distribution of the level of the MDR-1 within the cells of the

population. One would predict that the number of cells with high MDR-1 would increase with each cycle of therapy. The nadir and duration of the nadir at each dose level will be compared with the percentage of cells which are retroviral MDR-1 positive. This will test if the degree to which the marrow is replaced with MDR-1 positive cells will determine the resistance to Taxol.

RT PCR will be conducted on cells collected after transduction to obtain a population average for the level of expression of the retroviral MDR-1. RT PCR will also be conducted on a colony-by-colony basis using methylcellulose for this analysis. Cells will be taken from the peripheral blood and marrow, cultured in Dexter culture, and then plated in methylcellulose, or plated directly in methylcellulose. This will be repeated after each cycle of Taxol, and following recovery of marrow function following the autologous transplant.

We will also distinguish between the MDR-1 which arises from the endogenous genes, and from the retroviral transgenome. There will be two 5′ primers: one which is homologous to sequences in the 5′ untranslated region of the endogenous MDR- 1 mRNA, which are not present in the 5′ untranslated residual viral sequences which appear in the MDR-1 primary transcript. The 3′ primer will originate from DNA within the first exon of the MDR-1 transcription unit. Thus, the length of the product and the type of DNA in each primer product will permit discrimination of the endogenous and the retroviral gene product. Thus, we will be able to identify the level of expression of the endogenous and exogenous MDR-1 throughout the exposure to Taxol.

In order to determine if the introduction of MDR-1 into the cells of the marrow has slowed down the rate of recovery, we will compare the rate of recovery of the white blood cell count seen in autologous bone marrow transplant patients when advanced breast or other cancer patients are given the thiotepa, cyclophosphamide preparative regimen outlined in our protocol, when given an engrafting dose of CD34 selected autologous hematopoietic cells. We and others have now had experience with CD34 selected cells as reconstituting populations following intensive systemic preparative regimens, which are identical to the one we will use in this MDR-1 study. The median day to 500 and 1000 PMN/cu mm for CD34 transplants in heavily pretreated epithelial neoplasm patients is 17 days (range 10-22). If a delay in hematopoietic recovery occurs following transplant with the MDR modified marrow, we will be able to detect this if three patients or more exhibit a delay of recovery to greater than 40 days to a PMN count of $500/mm^3$.

Schema for Treatment Plan:

I. Non-ablative dose chemotherapy (cyclophosphamide, 4 gms/m^2) to recruit early precursor cells into a proliferative phase and to mobilize them into the peripheral blood.

II. A total of 4×10^8 nucleated cells/kg of peripheral blood cells for breast cancer patients and an engrafting dose of 20,000 CFUGM/kg will be collected. One half of it will be concentrated by the COBE pheresis instrument CD34 selected, and transduced as described in Appendix D. There will be at least a total of one million CD34 peripheral blood stem per kilogram of body weight for the transplant which will be composed of modified and unmodified cells.

III. Exposure of the peripheral blood collection which was CD34 selected to a safety-modified retrovirus which contains a MDR-1 cDNA will be undertaken following the protocol outlined in Appendix D. The peripheral blood or marrow back up collection which was not CD34 selected will be frozen in unmodified form. PCR assay for the MDR cDNA will be performed following the transduction using the assay outlined above. The PCR assay will be used to identify if the transduction was successful. A transduction frequency of 1% will be considered successful. The marrow will be frozen away using programmed freezing, as a backup for the peripheral blood transplant. The viability of the frozen marrow will be tested using the CFUGM assay.

IV. Preparative therapy will be delivered a single time before the transplantation and will involve cyclophosphamide, BCNU and thiotepa, in doses outlined earlier in this protocol. The administration of the preparative therapy will not begin until the patient has recovered from all of the toxicities of the non ablative dose therapy, and until the total white cell count is 2,000/cu mm, and the ANC is 1,800/cu mm, and until the hemoglobin is 8 gm/dcl, and the platelet count is 100,000/cu mm. The preparative therapy must also be delayed until all of the quality control data for the retroviral modification of the cells, and for the determination of the integrity of the hematopoietic cells stored is determined. This usually requires 3-4 weeks between the completion of the collection of the hematopoietic cells and the initiation of the preparative therapy.

V. Infusion of the peripheral blood with collection of a sample for RT PCR for MDR-1 of the thawed specimen before infusion.

VI. PCR for MDR-1 and functional assays for MDR-1 on the hematopoietic cells following a recovery of an ANC of 2000/mm^3 is achieved.

VII. Delivery of 12 courses of relatively intensive but not ablative levels of Taxol chemotherapy, at a starting dose of 60 mg/m^2 every three to four weeks, and the dose will be increased as follows (60, 120, 180, 225 and 275 mg/m^2) as is allowed by the rate of hematopoietic recovery. PCR assays will be conducted to assess the percentage of hematopoietic cells which are positive for the MDR-1 cDNA after each course of chemotherapy.

VIII PCR for MDR-1 on colonies of peripheral blood and marrow hematopoietic cells every three to four weeks following each course of therapy, as long as that therapy is administered, or until relapse, at which time the therapy of Taxol maintenance will no longer be administered. In situ functional analysis of the number of cells in peripheral blood and marrow by rhodamine efflux will be performed at the same time as RT PCR of MDR-1 to enumerate the number of cells with increased MDR-1 in the marrow and peripheral blood. After discontinuation of Taxol therapy, the PCR assays will be conducted every three months for two years, and every six months until a 5 year period has elapsed following the initial transplantation of modified marrow.

6.0 PRE-TREATMENT EVALUATION

6.1 A complete history and physical examination including documentation of all measurable disease, especially involvement of the peritoneum and other site of initial bulk disease which were established initially. A dental examination is also recommended.

6.2 Laboratory studies include CBC, platelet count, differential, SMA 12, liver and renal function tests, coagulation studies (PT, PTT, Fibrinogen, FSP), and cytogenetics. RT PCR for MDR-1 will be conducted as outlined above.

6.3 Bone marrow aspirate and biopsy for morphology, pathology, and cytogenetics. Immunohistochemical analysis for the presencer of breast cancer cells will be conducted as outlined in Appendix G. In addition, a bone scan will be conducted for the presence of bone marrow involvement.

6.4 An EKG and CXR will be performed on all patients. A urinalysis will also be obtained before therapy.

6.5 Pulmonary function studies with diffusion capacity.

6.6 A CT scan will be conducted to evaluate the extent of disease.

EVALUATION DURING STUDY (SEE APPENDIX F)

7.1 CBC, platelet, differential every 1-2 days during the initial induction.

7.2 SMA 12, electrolytes every three to seven days and as indicated additionally.

7.3 Bone marrow aspirate and biopsy for morphologic pathology, cytogenetics, and PCR for MDR-1 should be performed at marrow recovery (when ANC > 2000/cu mm).

7.4 Upon marrow recovery after transplant, a full work-up including CBC, platelet count and differential, SMA 12, and marrow studies including cytogenetics will be performed. Studies at remission will include CBC, platelet count, differential and SMA 12 every 4 weeks, marrow studies with cytogenetics every 6 months and as indicated by disease status.

A CBC will be conducted TIW during the period following each course of Taxol in order to determine the depth and duration of the nadirs following each course of Taxol.

7.5 Disease status will be evaluated after recovery from autologous bone marrow transplant, but before the initiation of the Taxol therapy so as to be able to separate the effect of the transplant from the effect of the post transplant Taxol on the disease status. This will involve a CT scan of sites which are known to be involved with breast cancer. Twelve courses of Taxol will be given in the event that no residual disease is detectable following the transplant.

7.6 The following will be collected before and after vector exposure and after hematopoietic recovery and on a six monthly basis for

five years after transplant: 2 x 10^6 cells for MDR-1 PCR, 2 x 10^6 blood and marrow cells for viral PCR, 2 x 10^6 cells for NIH3T3 viral amplification studies, 1 x 10^7 cells for Southerns and 10 cc of a red top tube for Western blots for viral antibodies.

7.7 At the time of relapse, samples of the tumor, obtained as part of staging procudures used to document the relapse, will be analyzed for the presence of the viral MDR-1 by PCR assay.

8.0 CRITERIA FOR RESPONSE AND TOXICITY (SEE APPENDIX E)

8.1 Criteria for toxicity will be as follows: Failure of the autologous cells to engraft to 500 PMN/cu mm by 30 days and the need to use back-up collections of hematopoietic cells from the marrow or peripheral blood for reconstitution.

8.2 Disordered hematopoiesis, abnormal morphology and alteration of the ratio of myeloid to erythroid cells will be established by morphology every three months as outlined in Appendix F.

8.3 Neurotoxicity or mucositis (Grade IV) in 3 consecutive patients will be grounds for decreasing the Taxol dose.

8.4 We will use NCI Investigational Agent Drug Reporting Criteria (see chart in Appendix E) and will report any Grade IV toxicity on this protocol to the FDA as well as to the Protocol and Information Office (Executive Plaza, North, Room 730, Bethesda, Maryland 20892).

8.5 CRITERIA FOR RESPONSE

All tumor measurements must be recorded and must have the longest diameter and its perpendicular, applied at the widest portion of the tumor, recorded. The antitumor activity of the drug under study will be evaluated according to the following response criteria:

a) Complete Remission (CR): Disappearance of all clinical evidence of active tumor for a minimum of 4 weeks.

b) Partial Remission (PR): 50% or greater decrease in the sum of the products of the diameters of measured lesions. No simultaneous increase in the size of any lesion or the appearance of new lesions may occur. This improvement must continue for 4 weeks to be considered a partial remission.

c) Minor Change: Same as partial remission but tumor regression is <50%.

d) No Change (NC): No change.

e) Progressive Disease (PD): Increase of at least 25% in the size of any measured lesion or appearance of one or more new lesions.

f) Minor change, no change and progressive disease will be considered as failure to therapy.

9.0 CRITERIA FOR REMOVAL FROM STUDY

9.1 Patients whose peripheral blood is not successfully marked with the MDR-1 retroviral vector, and whose peripheral blood cells do not exhibit an increase in the level of resistance to the non ablative dose chemotherapy with MDR drugs will not be continued on study unless there are preparations of marrow which contain elevated levels of hematopoietic cells which are resistant to MDR drugs above the levels which are encountered with unmodified cells.

Patients with unacceptable toxicity will be taken off study. Patients exhibit two consecutive grade IV hematopoietic or other toxicities on post transplant Taxol despite dose reductions, will be removed from the study. Patients who refuse further participation in the study will also be discontinued from the study. All other patients will continued to be followed as described above.

9.2 Patients with progressive disease will be removed from the study.

10.0 STATISTICAL CONSIDERATIONS

10.1 For this pilot trial, evaluation of the persistence of the functional state of the MDR sequences introduced into peripheral blood cells which are then transplanted will be conducted by aspiration of marrow cells following hematopoietic recovery from the transplant, and following each dose of post-transplantation therapy with Taxol for the first two years, or until the therapy is discontinued due to progression of disease, or until the death of the patient. PCR for MDR-1 will be used, and functional assays for the efficiency of efflux pumping by the marrow cells of dye rhodamine, and of the MDR drugs such adriamycin, and by study of in vitro resistance of the modified cells. The PCR assay will be done following the schedule outlined in Appendix F. The number of patients necessary to be entered for this program is between 10-20, so that a total of 10 evaluable patients can be transplanted and followed through at least 12 courses of Taxol. One patient per month will be evaluated and then study completed in one to two years. Success of the trial will be measured by decreases detactable in the duration and depth of the nadirs following Taxol chemotherapy during the dose escalation, which can be correlated with increases in the percentages of the cells which contain the MDR-1 viral transgene, by the statistical methods described in Appendix I.

10.2 Evaluation of the response of the breast cancer to the therapy delivered as preparative therapy before bone marrow transplantation, and after the post-transplantation therapy of cyclical high dose Taxol, as outlined in the Treatment Plan.

10.3 Stopping Rules: If three successive treatment deaths occur, or the mortality is >50% in the analyses of the cohorts at 6, 8 and 10 patients, the trial will stop. In addition, if 3 successive patients fail to engraft (failure to reach 500 neutrophils/per mm in 30 days post transplant), and delayed hematopoietic recovery, the trial will be stopped. If the tumor progresses during the preparative therapy or during Taxol therapy in an individual patient, the therapy will be stopped for that patient. If unacceptable levels of non-hematopoietic toxicity is encountered,

the Taxol will be stopped for that patient. If Grade IV hematopoietic toxicity is encountered in two successive courses of Taxol despite does reductions of Taxol in between, the Taxol despite dose reduction will be stopped for that patient.

10.4 Reporting Requirements: Reports will be submitted to the NIH RAC every 6 months and to CTEP on the CTEP ADU forms every 4 months. Serious adverse events will be reported to the NIH RAC, to the CTEP, and to the FDA.

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18. Deisseroth, A., Kantarjian, H., Talpaz, M., Champlin, R., Reading, C., Andersson, B., and Claxton, D. Human Gene Therapy 2:359-376, 1991.
19. Moore, K.A., Deisseroth, A.B., Reading, C.L., Williams, D.E., and Belmont, J.W. Blood 79:1393-1399, 1992.

The University of Texas
M.D. ANDERSON CANCER CENTER

INFORMED CONSENT

PROTOCOL TITLE: USE OF MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF BREAST CANCER: A PILOT TRIAL

1. _____ _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other informed consents you may have signed in other protocols.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY: This is a clinical research study to test whether normal peripheral blood stem cells can be made resistant to chemotherapy and thereby reduce the complications of chemotherapy.

3. DESCRIPTION OF RESEARCH: (A) Patients who agree to participate in this study will first receive cyclophosphamide chemotherapy, which may reduce the amount of breast cancer in the body, and at the same time prepare such patients for collection of the peripheral blood cells. Then, 250 cc (8 oz) of the blood will be withdrawn and run through a pheresis machine, which will separate peripheral blood cells from the plasma, which will be returned. To obtain bone marrow cells, general anesthesia will be given and cells will be extracted through needles which are placed in the hip bone.

(B) The peripheral blood cells will then be made more resistant to chemotherapy by introducing a gene for multi-drug resistance (called MDR-1) into the cells. The MDR-1 gene will be inserted into the cells by a virus which has been modified so that it should not cause an infection. This will be done by incubating the stem cells in the presence of the virus that carries the MDR-1 gene. Patients for whom the bone marrow or peripheral blood cell harvest is inadequate, or whose cells are not modified by the MDR-1 gene at an adequate rate, will not be eligible for transplant.

(C) Three chemotherapy drugs will be given (cyclophosphamide, BCNU, and thiotepa) to eliminate bone marrow, and to reduce the level of cancer in the body. This chemotherapy may also significantly reduce the amount of breast cancer in the body. The modified marrow cells which carry the MDR-1 gene will then be given by vein in

a manner which is similar to any blood transfusion. The recovery of full marrow function (with normal blood counts) will require 3-4 weeks in the hospital. During this time, IV fluids and antibiotics may be necessary.

(D) When the bone marrow (with the genetically-modified cells) has recovered to a normal level, Taxol will be given by intravenous infusion. Taxol is a drug which is effective against breast cancer, particularly at the late stages. Although the Taxol is expected to kill tumor cells, it is possible that the bone marrow cells that have been modified by the MDR-1 gene will be protected from being killed by the Taxol. The dose of Taxol which will be administered every three to four weeks will depend on how rapidly the white blood cell counts recover after each infusion.

(E) The patients participating on this trial will be followed for 5 years in remission or until relapse. 15-20 participants will be entered in this trial. In case of death, the patient's family will be asked for an autopsy because an accurate documentation of the precise cause of death may be important to all future patients.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

The side effects of the cyclophosphamide, BCNU, and thiotepa include fever, loss of hair, nausea and vomiting, infections, mouth sores, lung failure, cardiac failure, diarrhea and bladder inflammation, discoloration of the skin, skin ulceration, and bleeding. The side effects of Taxol include suppression of marrow function and neurological changes. The treatment related risk of death from the cyclophosphamide is estimated to be less than 5%. It is possible that the toxicity of the Taxol could be increased immediately following the transplant, but the dose of Taxol has been reduced to minimize this toxicity.

Risks, however, which may not yet have been identified, are possible. These are "theoretical risks" since they have not yet been seen. It is possible that the virus could change the cells which are marked so as to grow in an abnormal pattern, and even cause cancer or leukemia.

4a. This clinical research may involve unforeseeable risks to the participant (or to the embryo or fetus, if the participant is or may become pregnant during treatment). To help prevent injury to unborn children, upon recommendation by the attending physician, the participants should practice adequate methods of birth control throughout the period of their involvement in this clinical research study.

5. POTENTIAL BENEFITS: The use of chemotherapy resistant cells may potentially allow the use of higher doses of chemotherapy, therefore increasing the chances to destroy the breast cancer cells.

6. ALTERNATE PROCEDURES OR TREATMENTS: Participation in this marking study is voluntary. Other treatment options for this disease include conventional dose chemotherapy without modified cells. Taxol is a drug which is moderately active for the

treatment of breast cancer without the MDR-1 genetic modification and transplantation outlined in this program of therapy and patients can recieve the taxol therapy without participating in this program.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the investigational drug regimen involved and the investigator has been willing to reply to my inquiries. This investigational drug regimen will be administered under the above numbered, titled and described clinical research protocol at this institution. I hereby authorize Dr. _____ _____, the attending physician/investigator and designated associates to administer this investigational drug regimen.

8. I have been told and understand that my participation is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

   Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or effects of my decision with my physician.

   In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatment which may be of benefit at that time.

   I will be informed of any new findings developed during the course of this clinical research study, which may relate to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration and the National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for such injury.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of the clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of the study, I understand that I may be required to cover the cost of subsequent doses.

    Costs related to my medical care including expensive drugs, tests or procedures that may be specifically required by this clinical research study shall be my responsibility. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. I understand that refraining from breast feeding and practicing effective contraception are medically necessary and a prerequisite for my participation in this clinical research study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.

13. I may discuss questions or problems during or after this study with Dr. Albert Deisseroth at (713) 792-8750. In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman of the Surveillance Committee at (713) 792-3220 and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based on the above, I consent to participate in the research and have received a copy of the consent form.

_____	_____
DATE	SIGNATURE OF PARTICIPANT
_____	_____
WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR	SIGNATURE OF PERSON RESPONSIBLE AND RELATIONSHIP

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe that the participant understood this explanation.

_____
PHYSICIAN/INVESTIGATOR

FF Principal Investigator/Program Director(*Last,first,middle*): Deisseroth, Albert, B.

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Albert B. Deisseroth, M.D., Ph.D.	Professor and Chairman

EDUCATION(*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Univ. of Rochester, Rochester, NY	A.B.	1963	Chemistry
Univ. of Rochester School of Medicine, NY	Ph.D.	1968	Biochemistry
Univ. of Rochester School of Medicine, NY	M.D.	1970	Medicine
Intern & Junior Resident, Beth Israel Hospital, MA	Resident	1970-1972	Internal Medicine
Sidney Farber Center	Fellow	1975-1976	Hematology/Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

POSITIONS HELD

1970 - 1972	Intern and Resident, Beth Israel Hospital, Boston, MA
1972 - 1974	Clinical Associate, NHLI, NIH
1974 - 1975	Chief Resident Physican, NHLI, NIH
1975 - 1976	Clinical Fellow, Hematology/Oncology, Children's Hospital Medical Center, Boston, MA
1976 - 1981	Head, Experimental Hematology Section, Pediatric Oncology Branch, NCI, NIH
1981 - 1987	Chief, Hematology/Oncology Section, Department of Medicine, UCSF Service, San Francisco VA Medical Center, and Professor of Medicine, UCSF
1987 - present	Prof. of Medicine and Chairman, Dept. of Hematology, U.T. M.D. Anderson Cancer Center, Houston, TX
1987 - present	Anderson Professor of Cancer Treatment and Research, M.D. Anderson Cancer Center, Houston, TX

RELEVANT PUBLICATIONS: (Selected from 247 publications)

1. Wedrychowski A, Howard OMZ, Seong D, Paslidis N, Talpaz M, Kantarjian H, Hester J, Turpin J, Lopez-Berestein G, Gutterman J, Freireich E, and Deisseroth A: Alpha-interferon alters binding of 84-, 50-, and 95-kDa nuclear proteins from CML patients to interferon-inducible transcriptional enhancers. J Biol Chem 265:21433-21440, 1990.
2. Seong DC, Sims S, Johnson E, Howard OMZ, Hester J, Talpaz M, Kantarjian H, and Deisseroth A: A phosphatase activity present in peripheral blood myeloid cells of CML patients but not normal individuals alters nuclear protein binding to transcriptional enhancers of interferon-inducible genes. J Clin Invest 86:1664-1670, 1990.
3. Wallerstein R Jr, Spitzer G, Dunphy F, Huan S, Hortobagyi G, Yau J, Buzdar A, Holmes F, Theriault R, Ewer M, LeMaistre CF, Dicke K, and Deisseroth A: A phase II study of mitoxantrone, etoposide (VP-16), and thiotepa (MVT) with autologous marrow support for patients with relapsed breast cancer. J Clin Oncol 8:1-4, 1990.
4. Estey EH, Dixon D, Kantarjian H, Keating MJ, McCredie K, Bodey GP, Kurzrock R, Talpaz M, Freireich EJ, Deisseroth AB, and Gutterman JU: Treatment of poor-prognosis, newly-diagnosed acute myeloid leukemia with Ara-C and recombinant human granulocyte-macrophage colony-stimulating factor. Blood 75:1766-1769, 1990.
5. Seong D, Sims S, Johnson E, Lyding J, Lopez A, Garovoy M, Talpaz M, Kantarjian H, Lopez-Berestein G, Reading C, and Deisseroth A: Activation of class I HLA expression by TNF-alpha and gamma-interferon is mediated through protein kinase C-dependent pathway in CML cell lines. Br J Haematol 78:359-367, 1991.
6. Claxton D, Suh S-P, Filaccio M, Ellerson D, Gaozza E, Andersson B, Brenner M, Reading C, Feinberg A, Moen R, Belmont J, Moore K, Talpaz M, Kantarjian H, and Deisseroth A: Molecular analysis of retroviral transduction in chronic myelogenous leukemia. Human Gene Therapy 2:317-321, 1991.
7. Etkin M, Filaccio M, Ellerson D, Suh S-P, Claxton D, Gaozza E, Brenner M, Moen R, Belmont J, Moore KA, Mosely AM, Reading C, Khouri I, Talpaz M, Kantarjian H, and Deisseroth A: Use of cell-free supernatants of safety-modified viruses for transduction of cells from the marrow of chronic phase and blast crisis CML patients and from normal individuals. Human Gene Therapy 3:137-145, 1992.

8. Moore K, Reading C, Deisseroth A, William D, and Belmont J: Stromal support enhances cell-free retroviral vector transduction of human bone marrow long term culture initiating cells. Blood 79:1393-1399, 1992.
9. Wedrychowski A, Henzel W, Huston L, Paslidis N, Ellerson D, McRae M, Seong D, Howard OMZ, and Deisseroth A: Identification of proteins binding to interferon-inducible transcriptional enhancers in hematopoietic cells. J Biol Chem 267:4533-4540, 1992.
10. Johnson E, Henzel W, and Deisseroth A: A protein disulfide isomerase isolated from chronic myelogenous leukemia cells alters complex formation between nuclear proteins and regulatory regions of interferon-inducible genes. J Biol Chem 267:14412-14417, 1992.
11. Sims S, Cha Y, Romine M, Gao P-Q, Gottlieb K, and Deisseroth A: A novel interferon-inducible domain: Structural and functional analysis of the human IRF-1 gene promoter. Mol Cell Biol 13:690-702, 1993.
12. del Giglio A, O'Brien S, Ford R, Saya H, Manning J, Keating M, Johnston D, Khetan R, El-Naggar A, and Deisseroth A: The prognostic value of proliferative cell nuclear antigen expression in chronic lymphoid leukemia. Blood 79:2717-2720, 1992.
13. Zhang W, Drach J, Andreeff M, and Deisseroth A: Proliferation of hematopoietic cells is accompanied by suppressed expression of heat shock protein 70. Biochem Biophys Res Comm 183:733-738, 1992.
14. Cha Y, Sims S, Romine M, Kaufmann M, and Deisseroth A: Human interferon regulatory factor-1: Intron/exon organization. DNA and Cell Biology 11:605-611, 1992.
15. Zhang W, Hittelman W, Van N, Andreeff M, and Deisseroth A: The phosphorylation of retinoblastoma gene product in human myeloid leukemia cells during the cell cycle. Biochem Biophys Res Comm 184:212-216, 1992.
16. Zhang W, Hu G, and Deisseroth A: Polymorphism at codon 72 of the p53 gene in human acute myelogenous leukemia. Gene 117:271-275, 1992.
17. Hu G, Zhang W, and Deisseroth A: P53 gene mutations in acute myelogenous leukemia. Br J Haematol 81:489-494, 1992.
18. Kantarjian HM, Talpaz M, Kontoyiannis D, Gutterman J, Keating MJ, Estey EH, O'Brien S, Rios MB, Beran M, and Deisseroth A: Treatment of chronic myelogenous leukemia in accelerated and blastic phases with daunorubicin, high-dose cytarabine, and granulocyte-macrophage colony stimulating factor. J Clin Oncol 10: 398-405, 1992.
19. Claxton DF, Reading CL, Nagarajan L, Tsujimoto Y, Andersson B, Estey E, Cork MA, Huh YO, Trujillo J and Deisseroth A: Correlation of CD2 expression with PML gene breakpoints in patients with acute promyelocytic leukemia. Blood 80:582-586, 1992.
20. Kornblau S, Xu H-J, del Giglio A, Hu S-X, Zhang W, Smallwood L, Beran M, Estey E, Andreeff M, Trujillo J, Cork MA, Smith TL, Benedict WF, and Deisseroth A: Clinical implications of altered retinoblastoma protein expression in newly diagnosed and relapsed acute myelogenous leukemia. Cancer Res 52:4587-4590, 1992.
21. Zhang W, Hu G, Estey E, Hester J, and Deisseroth A: Altered conformation of the p53 protein in myeloid leukemia cells and mitogen-stimulated normal blood Cells. Oncogene 7:1645-1647, 1992.
22. Estey E, Thall PF, Kantarjian H, O'Brien S, Koller CA, Beran M, Gutterman J, Deisseroth A, and Keating M: Treatment of newly diagnosed acute myelogenous leukemia with granulocyte-macrophage colony-stimulating factor (GM-CSF) before and during continuous-infusion high-dose ara-C + Daunorubicin: Comparison to patients treated without GM-CSF. Blood 79:2246-2255, 1992.
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24. Gao P-Q, Sims SH, Chang DC, and Deisseroth AB: Interferon-gamma priming effects in the activation and deactivation of ISGF3 in K562 cells. J Biol Chem 268:12380-12387, 1993.
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26. Zhang W, Cork A, Trujillo J, Calvert L, Hu G, Andersson B, and Deisseroth AB: Mutations of the p53 gene are associated with genetic instability and immortalization in myelogenous leukemia. Cancer Res Therapy Control 3:139-144, 1993.
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29. Kornblau SM, Chen N, del Giglio A, O'Brien S, and Deisseroth AB: Retinoblastoma protein expression is frequently altered in chronic lymphocytic leukemia. Cancer Res 54:242-246, 1994.
30. Cha Y and Deisseroth AB: Human interferon regulatory factor 2 gene: intron-exon organization and functional analysis of 5' flanking region. J Biol Chem 269:5279-5287, 1994.
31. Hanania EG and Deisseroth AB: Serial transplantation shows that early hematopoietic precursor cells are transduced by MDR-1 retroviral vector in a mouse gene therapy model. In Press, Cancer Gene Therapy, 1994.
32. Deisseroth AB, Zu Z, Claxton D, Hanania EG, Fu S, Ellerson D, Goldberg L, Thomas M, Janicek K, Anderson WF, Hester J, Korbling M, Durett A, Moen R, Berenson R, Heimfeld S, Brenner M, Hamer J, Calvert L, Tibbits P, Talpaz M, Kantarjian H, Champlin R, and Reading C: Genetic marking shows that Ph + cells present in autologous transplants of chronic myelogenous leukemia contribute to relapse after autologous bone marrow in CML. In Press, Blood, 1994.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Richard E. Champlin, M.D.	Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Purdue University, Indiana	B.S.	1971	Engineering Science
University of Chicago, Illinois	M.D.	1975	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

EMPLOYMENT:

1975-1978	UCLA Harbor General Hospital, Internship and Residency in Medicine
1978-1981	UCLA Center for the Health Sciences Fellowship in Hematology and Oncology
1981-1985	Assistant Professor of Medicine, UCLA School of Medicine
1985-1990	Associate Professor of Medicine, UCLA School of Medicine
1983-1990	Director, Transplantation Biology Program UCLA Johnsson Comprehensive Cancer Center
1985-1990	Director, UCLA Hematology/Oncology Fellowship Program
1990-present	Professor of Medicine, University of Texas, M.D. Anderson Cancer Center Chief, Section of Bone Marrow Transplantation
1990-present	President, Council of Donor Transplant and Collection Centers, National Marrow Donor Program
1990-present	Member of Executive Board of Directors, International Bone Marrow Transplant Registry
1991-present	Consulting Staff, The Methodist Hospital, Internal Medicine and Hematology/Oncology

PUBLICATIONS:

1. Champlin RE, Gale RP. Acute lymphoblastic leukemia: recent advances in biology and therapy. Blood 1989; 73:2051-2066.
2. Sawyers CL, Timson L, Kawasaki ES, Clark SS, Witte ON, Champlin R. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci USA. 1990;87:563-567.
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7. Champlin RE, Ho WG, Gajewski J, Feig S, Burnison M, Holley G, Greenberg P, Lee K, Schmid I, Giorgi J, Yam P, Petz L, Winston D, Warner N, Reichert T. Selective depletion of CD8 positive T-lymphocytes for prevention of graft-versus-host disease following allogeneic bone marrow transplantation. Blood 1990; 76:418-423.

8. Champlin RE, Gajewski J, Nimer S, Vollset S, Landaw E, Winston D, Schiller G, Ho W. Post-r chemotherapy for adults with acute myelogenous leukemia: improved survival with h cytarabine/daunorubicin consolidation treatment. J Clin Oncol 1990; 8:1199-1206.
9. Champlin RE, Ho WG, Nimer SD, Gajewski JG, Selch M, Burnison M, Holley G, Yam P, Winston DJ, et al. Bone marrow transplantation for severe aplastic anemia. Effect of a pre regimen of cyclophosphamide-low-dose total-lymphoid irradiation and posttransplant cyclo methotrexate therapy. Transplantation 1990; 49:720-724.
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12. Ugozzoli L, Yam P, Petz LD, Ferrara GB, Champlin RE, Forman SJ, Koyal D, Wall Amplification by the polymerase chain reaction of hypervariable regions of the human gen evaluation of chimerism after bone marrow transplantation. Blood 1991; 77:1607-1615.
13. Nimer SD, Golde DW, Kwan K, Lee K, Clark S, Champlin RE. In vitro production of gran macrophage colony-stimulating factor in aplastic anemia: Possible mechanisms of ac antithymocyte globulin. Blood 1991; 78:163-168.
14. Champlin RE. Immunobiology of bone marrow transplantation as treatment for hem malignancies. Transplant Proc 1991; 43:197-207.
15. Storb R and Champlin RE. Bone marrow transplantation for severe aplastic anemia. Bone Transplantation 1991; 8:69-72.
16. Champlin RE. Graft-versus-leukemia without graft-versus-host disease: an elusive goal of bone transplantation. Semin Hematol 1992; 29:46-52.
17. Schiller G, Gajewski J, Nimer S, Territo M, Ho W, Lee M, Champlin RE. A randomized s intermediate versus conventional dose cytarabine as induction chemotherapy for acute myel leukemia. Br J Hematol, 1992;in press.
18. Gluckman E, Horowitz M, Champlin R, Hows J, Bacigalupo A, Biggs J, Camitta B, Gale R, C Smith E, Marmont A, Masaoka T, Ramsay N, Rimm A, Rozman C, Sobocinski Km Speck B, Bo Bone marrow transplantation for severe aplastic anemia: influence of conditioning and graft-vers disease prophylaxis regimens on outcome. Blood 1992;79:269-275.
19. Horowitz M, Przepiorka D, Champlin RE, Gale RP, Gratwohl A, Herzig RH, Prentice HG, Rin Ringden O, Bortin M. Should HLA-Identical sibling bone marrow transplants for leukemia be re to large centers? Blood 1992; 79:2771-2774.
20. Lee M, Khouri I, Champlin RE, Kantarjian H, Talpaz M, Trujillo J, Freireich E, Deisseroth S. Detection of minimal residual leukemia by polymerase chain reaction of the bcr/abl transc Philadelphia positive chronic myelogenous leukemia following allogeneic bone marrow transpla Brit J Hematol 1992:(in press)

CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

FF Principal Investigator/Program Director (Last, first, middle):

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Gabriel N. Hortobagyi	Professor of Medicine 7/22/46 Chairman, Breast and Gynecologic Medical Oncology

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Universidad Natl of Colombia	M.D.	1970	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

POSTGRADUATE TRAINING AND EMPLOYMENT

1971-1974 Int Med Resident, St. Luke's Hospital, Cleveland, OH
1974-1976 Fellow Medical Oncology, U.T. M.D. Anderson Hosp, Houston
1976-1977 Faculty Associate, Medical Breast Service, UTMDACC
1977-1980 Assistant Professor of Medicine, UTMDACC
1980-1985 Associate Professor of Medicine, UTMDACC
1980-1985 Director, Fellowship Program, Dept. Med. Onc., UTMDACC
1984-1992 Chief, Breast Medical Oncology Service, UTMDACC
1985-pres Professor of Medicine, UTMDACC
1991-pres Nylene Eckles Professor of Breast Cancer Research, UTMDACC
1992-1993 Chairman, Breast and Gynecologic Medical Oncology, UTMDACC

CERTIFICATION

1975 Diplomat, American Board of Internal Medicine
1977 Diplomat, American Board of Internal Medicine (Medical Oncology)

HONORS

1977 Nylene Eckles Fellowship in Breast Cancer Research
1984 Mario Gaitan Yanguas Lecturer
1991 Nylene Eckles Professorship in Breast Cancer Research

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HUMAN GENE TRANSFER PROTOCOL

9406-078 Liu, Johnson, M. and Young, Neal S.; National Institutes of Health, Bethesda, Maryland; *Retroviral Mediated Gene Transfer of the Fanconi Anemia Complementation Group C Gene to Hematopoietic Progenitors of Group C Patients.*

Date of RAC Approval: 6-9-94
Date of NIH Approval: 2-12-94

Fanconi anemia (FA) is a rare genetic disorder characterized by progressive pancytopenia, congenital abnormalities, and a predisposition to malignancy. Therapy is currently limited to allogeneic marrow transplantation; patients lacking a suitable donor usually die from aplasia or acute leukemia. Recently, mutation in a novel gene named FACC (Fanconi anemia C-complementing) has been identified as causing one type of FA. FACC mutations, which introduce splicing errors or stop codons, have been identified in ~15% of FA patients. We have recently been successful in functional complementation of four FA cell lines using retroviral vectors to transfer a copy of the normal FACC gene. We also analyzed the ability of our viral vectors to functionally correct hematopoietic progenitor cells from a patient bearing a splice donor mutation. As for the lymphoid cell lines, these CD34-enriched cells were extremely sensitive to MMC. After infection of these progenitor cells with viral vectors bearing normal FACC, the progenitors gave rise to increased numbers of colonies both in the absence and presence of up to 5 nM MMC, whereas control cells were completely destroyed by 1 nM MMC. In summary, we have demonstrated that: (1) retroviral vectors can be engineered to transfer a normal FACC gene to FA(C) lymphoid cell lines and primary hematopoietic cells; (2) introduction of a normal FACC gene into CD34+ progenitors markedly enhances their growth in the absence and presence of MMC.

This study is designed to determine whether hematopoietic progenitors transduced with the normal FACC gene can be reinfused safely into FA(C) patients. CD34+ cells obtained from G-CSF mobilized peripheral blood will be transduced *ex vivo* over a 72-hour period in the presence of IL-3, IL-6, and Stem Cell Factor with the FACC retroviral vector. These transduced cells will be reinfused into FA(C) patients. Patients will be monitored for toxicities as well as evidence of successful gene transfer and expression. The procedure will be repeated up to a total of 4 times with each treatment 2-4 months apart. Theoretically, these rescued stem cells should have a selective growth advantage within the hypoplastic FA marrow environment *in vivo*.

Fanconi's anemia is an inherited disorder that can produce bone marrow failure. In addition, some patients with Fanconi's anemia have physical defects, usually involving the skeleton or kidneys. The major problem for most patients is aplastic anemia. In aplastic anemia, the blood counts for red blood cells, white blood cells, and platelets are low because the bone marrow fails to produce these cells. Some patients with Fanconi's anemia also can develop leukemia or cancers in other organs. Many laboratory studies have suggested that Fanconi's anemia is due to an inherited defect in the ability of cells to repair DNA. Recently, the gene for one of the four types of Fanconi's anemia, type C, has been identified. It is known that this gene is defective in patients with Fanconi's anemia type C.

Studies in our laboratory have suggested that Fanconi's anemia type C may be a good candidate disease for gene therapy. We have placed the normal Fanconi's anemia type C gene into a retroviral vector and introduced the gene into cells, derived from Fanconi's anemia type C patients. In the laboratory, when we have compared cells lines and bone marrow cells from Fanconi's anemia patients before and after this procedure, we see a return towards normal of cell growth, resistance to the chemical agents that harm Fanconi's anemia cells, and a more normal appearance of the cells' chromosomes. Because the cells containing the normal Fanconi's anemia type C gene grow better, these cells should have a competitive advantage compared to unaltered Fanconi's anemia type C cells.

The purpose of this research protocol is to test whether we can safely introduce the normal Fanconi's anemia type C gene into stem cells of patients with this disease. Stem cells are the cells in the bone marrow and blood that give rise to the white cells, platelets, and red cells. We will treat blood cells from patients with Fanconi's anemia type C using the vector containing the Fanconi's anemia gene in a test tube and then return these cells to patients. If the cells are genetically altered, we expect to be able to detect the normal gene in blood and bone marrow cells afterwards. We hope that cells that contain the Fanconi's anemia gene will grow well in the bone marrow, and that we will be able to detect normal stem cells in special tissue culture studies. If we are successful, we should also be able to correct the chromosome abnormality. It may even be possible to increase blood counts in patients with Fanconi's anemia using this procedure. However, it should be clear that the major purpose of this protocol is to test the safety of this technique and to determine whether we can transfer the Fanconi's anemia gene successfully.

In order to obtain sufficient stem cells from patients with Fanconi's anemia, we will treat each patient with a hematopoietic growth factor called G-CSF or granulocyte-colony stimulating factor. G-CSF will be given for one week, at the end of which time blood cells will be removed by a process called apheresis for treatment with the Fanconi's anemia type C gene vector.

RETROVIRAL MEDIATED GENE TRANSFER OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C GENE TO HEMATOPOIETIC PROGENITORS OF GROUP C PATIENTS

Principal investigator(s):

Johnson M. Liu, M.D.
Senior Research Investigator
Hematology Branch, NHLBI
Bldg. 10, Rm. 7C103
Bethesda, MD 20892
Telephone (301) 496-5093
Telefax (301) 496-8396

Neal S. Young, M.D.
Chief
Hematology Branch, NHLBI
Bldg. 10, Rm. 7C103
Bethesda, MD 20892
Telephone (301) 496-5093
Telefax (301) 496-8396

Associate Investigators:

NIH/NHLBI:

Christopher E. Walsh, M.D., Ph.D.
Michele Cottler-Fox, M.D.
Charles Carter
Cynthia Dunbar, M.D.
Austin John Barrett, M.D.
Robert Emmons, M.D.
Lisa Esposito, R.N.

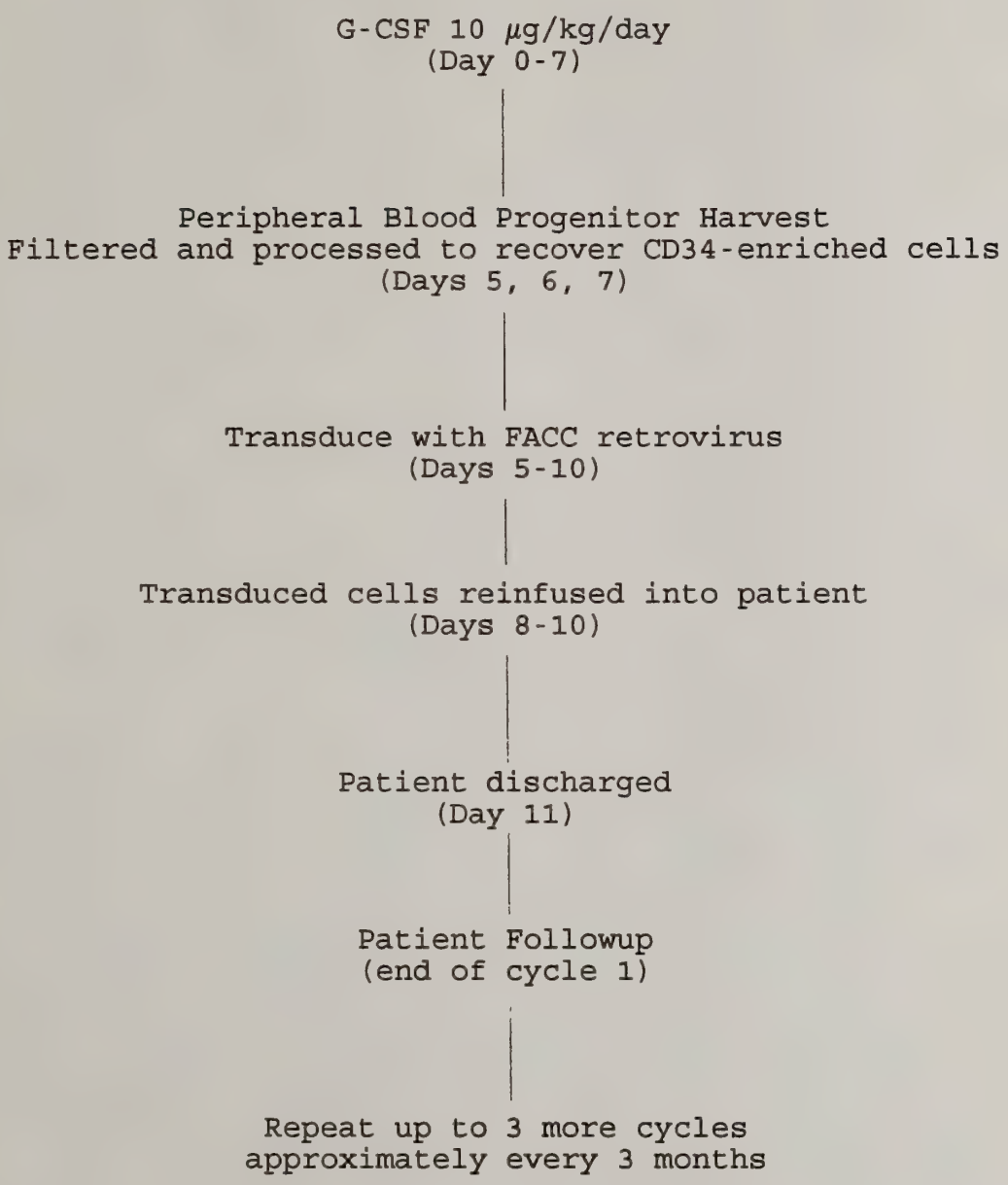
GTI Collaborator

Robert C. Moen, M.D., Ph.D.

PROTOCOL SCHEMATIC

Peripheral Blood Progenitor Harvest

Patients with Fanconi Anemia, Group C



1.0 BACKGROUND AND RATIONALE

1.1 Disease Background/Current Treatment

Fanconi anemia (FA) is an autosomal recessive genetic disorder characterized by progressive pancytopenia, congenital abnormalities, and a predisposition to malignancy(1). Most patients present at age 6-8 and die as young adults from complications of severe bone marrow aplasia or from the development of acute leukemia.

Therapy is currently limited to allogeneic bone marrow transplantation from a normal HLA-identical sibling(1). Unfortunately, the majority of FA patients will not have a suitable donor. Non-HLA-matched donors and HLA-matched unrelated donors could be used, but the morbidity and mortality risk is much greater than that seen with patients having an HLA-matched sibling donor.

Somatic cell methods have established that at least four different genes can cause FA(2). Recently, mutation in a novel gene named FACC (Fanconi anemia C-complementing) has been identified as causing one type of FA(3). The gene sequence shares no similarities to any known gene and does not possess known functional motifs. FACC maps to chromosome 9q and encodes a set of cDNAs that share the same coding region but differ at both 5' and 3' untranslated regions(2,3). The protein has a predicted size of 63 kD. FACC mutations, which introduce splicing errors or stop codons, have been identified in approximately 15% of FA patients(4).

1.2 Strategy of Gene Therapy

The hallmark of the FA cell phenotype is extreme sensitivity to bifunctional DNA cross-linking agents [such as mitomycin C (MMC) and diepoxybutane (DEB)] resulting in chromosomal instability and cell death(5). It is thought that this hypersensitivity is a result of a defect in the repair of DNA damage. Transfer of a normal FACC cDNA corrects the phenotypic defect, resulting in normalized cell growth in the presence of agents such as MMC. FA hematopoietic stem cells rescued by gene therapy should have a selective growth advantage within the hypoplastic marrow environment. For these reasons, we believe that a gene therapy strategy may be an effective treatment strategy for FA(C).

1.3 Description of Viral Vector

See appendix.

1.4 Preclinical

Retroviral mediated gene transfer in rodent and large animal transplant models

Extensive preclinical studies have documented the ability of retroviral vectors to transfer genes to the hematopoietic stem cells of mice(6-7). Since the target cells must be cycling to facilitate efficient gene transfer, growth factors are included in the infection mixture; in particular, stem cell factor (SCF), interleukin-3 (IL-3) and interleukin-6 (IL-6) have been shown to increase transduction

efficiencies(8).

Gene transfer has also been achieved in a number of large animal bone marrow transplantation models(7) Bone marrow stem cells of rhesus monkeys have been successfully transduced by two groups(9-11). Multilineage reconstitution of transduced cells was reported and long-term expression of the foreign gene achieved(10). For example, rhesus CD34+ cells have been transduced with the bacterial neomycin phosphotransferase(Neo^R) gene; autologous transplantation with these marked cells led to long-term reconstitution(11). Similar results have been obtained transducing rhesus CD34+ cells with a vector carrying the adenosine deaminase (ADA) gene (D. Bodine, personal communication). Hematopoietic stem cells from dogs(12) and cats(13) have also been successfully transduced with retroviral vectors.

Retroviral mediated transfer of FACC into FA(C) lymphoblasts

Transformed lymphoblast cell lines have been established from FA patients bearing mutations in FACC alleles. These mutations include a point transition(3), a nucleotide deletion(4), and a nonsense mutation(14). We tested the ability of our FACC retrovirus to transduce all three types of mutant lines. As expected, all lines were extremely sensitive to the cross-linking agent MMC. Cell lines transduced by the FACC retrovirus were able to proliferate at concentrations of MMC several orders of magnitude higher than parental controls (and equivalent to a line established from a normal individual). One genetically corrected cell line was tested for susceptibility to MMC-induced chromosomal breakage and was found to have been normalized on cytogenetic analysis. Southern analysis confirmed successful transduction of the normal FACC gene in these defective lines.

Retroviral mediated transfer of FACC into FA(C) CD34+ hematopoietic progenitors

We next analyzed the ability of the vectors to correct the functional growth defect in hematopoietic progenitor cells from a patient bearing a splice donor mutation in FACC. Progenitor cells were obtained by peripheral blood leukapheresis and purified by an immunoaffinity column to enrich for CD34 expression. As for lymphoid cell lines, these CD34-enriched cells were extremely sensitive to MMC. After infection with the FACC vector, these progenitors gave rise to markedly increased numbers of colonies both in the absence and presence of $\leq$5 nM MMC, whereas no colonies formed from control cells, even in the absence of MMC. PCR analysis confirmed the presence of proviral DNA in colonies derived from these CD34+ progenitors. Besides directly confirming the ability of our FACC virus to transduce target cells from a FA(C) patient, these studies suggest that FA stem cells can be rescued by gene therapy. The infection procedure is exactly

analogous to our proposal in this protocol and underscores the feasibility of this approach.

1.5 Clinical

Experience with retroviral mediated gene transfer into human marrow cells

Studies of retroviral gene transfer in relapsed patients undergoing autologous bone marrow transplantation (ABMT) for acute leukemia have provided evidence for successful marking of human hematopoietic progenitor cells(15 and M. Brenner, unpublished data). Patients have now been followed for up to one year after ABMT: analysis of these patients has documented gene transfer into all lineages of marrow-derived cells including neutrophils and lymphocytes. These studies employed vectors very similar to those to be used in this study and suggest that retroviral mediated gene transfer into very primitive hematopoietic progenitor cells is possible. Furthermore, since the marrow was not cultured in the presence of hematopoietic growth factors during the transduction procedure, the efficiency of gene transfer in these studies would be expected to be lower than might be achieved using methods proposed in this protocol.

Ability to transfer marrow progenitor cells into non-ablated recipients

In mouse models, investigators have demonstrated successful transplantation of marrow stem cells into recipient mice without first "making space" in the marrow environment with ablative treatments(16-19). Long-term chimeras up to two years post transplantation could be established without myeloablation. These experiments suggest that engraftment of autologous cells treated *ex vivo* with retroviral vectors may be possible without previous myeloablation. Further experience with transplantation for human immunodeficiency disorders such as severe combined immune deficiency (SCID) and Wiskott-Aldrich syndrome has supported the concept that prior myeloablation may not be necessary for engraftment of marrow cells.

Rationale

As outlined in the previous sections, we have demonstrated that our retroviral vector can transfer and express the FACC gene in the target population CD34-enriched progenitors of FA(C) patients. The level of expression was sufficient to "rescue" stem cells in our functional assay in a manner which should be directly analogous to our proposed protocol. Human and animal experience suggests that it should be possible to engraft hematopoietic stem cells without requiring ablation. Gene corrected stem cells would be expected to have a survival advantage within the hypoplastic FA marrow environment. All components necessary to execute the plan in this protocol have been demonstrated.

1.6 Objectives

1.61 Primary Objectives:

(a) To investigate whether the FACC gene can be safely transferred to hematopoietic progenitor cells by retroviral mediated gene transfer.
(b) To determine the extent of engraftment following autologous transplantation of transduced hematopoietic stem cells into FA patients without prior ablation of recipient marrow.

1.62 Secondary Objectives:
To investigate whether the transfer of a normal FACC gene into patients carrying defective FACC alleles will correct the cell phenotype and improve hematopoietic function.

2.0 STUDY DESIGN

2.1 Overview

This is a single site, unblinded pilot study of gene therapy in patients diagnosed with Fanconi anemia. The treatment will be retroviral mediated transfer of a normal FACC gene into peripheral blood progenitors (PBSC) from patients with defective FACC alleles. To mobilize PBSC prior to harvest, G-CSF will be administered for 7 days. PBSC will be collected on days 5, 6 and 7, and cells will be processed for enrichment of CD34+ cells. Each time that PBSC are collected they will be cultured and transduced with the FACC retroviral vector. Transduced cells will then be reinfused into the patient.

2.2 Number of Patients/Participating Sites

Six participants will be enrolled in this study.

2.3 Patient Management/Special Facilities

Interested potential study patients will be scheduled for a screening evaluation to determine their eligibility. Participants will then be hospitalized for approximately 10 days every two to four months for approximately one year. Patients will be monitored daily while being treated, monthly between cycles, and every 3 months during follow-up (as an outpatient). Patients will be reinfused with PBSC up to a total of 4 times (4 cycles). Monitoring will include evaluation for toxicity as well as response of hematopoietic function. Both DNA and RNA analyses will be used to document transfer and expression of the FACC gene.

2.4 Study Duration

Each PBSC cycle will take approximately two weeks to complete. Cycles will be repeated no more than every 2 months, and there may be up to 4 cycles total. Upon completion of study treatment, patients will be followed every 3 months for the first year, every 6 months for the next year, and yearly afterwards.

3.0 STUDY TREATMENT

3.1 Justification of Dosages

It is estimated that the ratio of total bone marrow cells to stem cells is 10^5:1. With this assumption, some 10^4 stem cells will be exposed to the FACC vector for each patient. If the transduction efficiency is as low as 1%, stem cell transduction should still be detectable in patients. Preclinical studies imply that

the transduction frequency into progenitor cells may be as high as 50%(20).

3.2 Formulation, Packaging, and Storage

More complete descriptions of these reagents are included in Appendix V. Brief descriptions are listed below:

G-CSF

Granulocyte colony stimulating factor is manufactured by Amgen. G-CSF is known to stimulate granulocytes and their precursors. It is used in this protocol to mobilize peripheral blood stem cells.

SCF

Stem cell factor is manufactured by Amgen. SCF acts synergistically with other growth factors to promote growth of early hematopoietic cells. It is used in this protocol to increase the efficiency of gene transfer into the earliest progenitor cells.

IL-3

Interleukin-3 is manufactured by Sandoz. IL-3 acts synergistically with a variety of growth factors to stimulate cell division of early marrow cells, and it is used in this protocol because its use has been shown to increase the efficiency of retroviral gene transfer.

IL-6

Interleukin-6 is manufactured by Sandoz. IL-6 acts synergistically with other growth factors to stimulate hematopoietic cell division and, in general, increases the efficiency of gene transfer.

FACC Retroviral Vector Supernatant

FACC retroviral supernatant is manufactured by Genetic Therapy, Inc. It consists of the conditioned medium obtained from producer cells. These producer cells have been engineered to produce retroviral vector particles with an amphotropic envelope so that the vector may be transferred into human cells.

3.3 Treatment Procedures

(Refer to section 6.0 for dose modification and toxicity management)

3.31 Harvest of Hematopoietic Stem Cells

1. Peripheral Blood Stem Cell Harvest:

   Peripheral blood stem cells will be harvested after mobilization with the growth factor G-CSF.

   Study Days 0-6: G-CSF will be given at a dose of 10μg/kg/day subcutaneously for a period of 7 consecutive days. The drug will be given as a single daily injection, and the patient may receive acetominophen or other non-aspirin analgesics for any possible associated fever or bone pain.

   Study Days 5-7: On days 5, 6, and 7, the patient will undergo apheresis procedures for the collection of peripheral blood mononuclear cell fractions with an expected maximum yield of 1 x 10^8 cells per kilogram body weight.

3.32 Separation of CD34+ Population of Hematopoietic Cells:

All the cells from the peripheral blood stem cell

harvest will be processed to remove contaminating red cells. Enrichment for CD34+ cells will be performed using the CellPro Stem Cell Separator. This procedure involves the incubation of the cells with a biotinylated murine anti-human CD34 antibody 12.8, washing the cells, and passing them over an avidin Biogel column. The cells adhering to the column will be collected after mechanical dislodgement. After enrichment, a sample of CD34+ cells will be saved for further analysis and use as pre-gene therapy controls for colony-forming and other assays.

3.33 Transduction and Transplantation Procedures:
The retroviral vector supernatant for this study will be produced by Genetic Therapy, Inc. (GTI). GTI has produced the retroviral vector supernatants used in most of the gene therapy studies performed thus far. The vector supernatant used in this protocol will be produced and quality control- tested in a similar fashion as for other retroviral vector protocols.

1. Transduction Procedure
After removal of aliquots of enriched cells for studies, the remainder of the CD34-enriched population of cells will be transduced with the retroviral vector in flasks. Separated CD34-enriched cells will be cultured (1×10^5/ml) in vector supernatant with IL-3, IL-6, and SCF and 4 μg/ml protamine sulfate for a period of 72 hours. Every 24 hours the cells will be pelleted and resuspended in fresh supernatant, growth factors, and protamine. After the period in culture, the cells will be washed and samples taken for analysis (gene transfer efficiency, colony-forming ability, assays for replication-competent retrovirus). The remaining cells will be resuspended in heparinized medium for reinfusion. Included in Appendix VI is a sample certificate of the tests that will be performed on each hematopoietic stem cell sample prepared for reinfusion into the patient.

2. Reinfusion of Transduced Cells
The transduced cells will be reinfused into the patient over a period of two hours or less. Premedication, given 30 min before infusion begins, will consist of 25 mg diphenhydramine IV and 650 mg acetominophen given po (100 mg hydrocortisone IV is optional). Vital signs will be monitored every 15 min up to two hours post-infusion.

3. Post-Infusion Care
Initially, patients will be monitored in the hospital overnight. If no adverse events are seen, the patients will be discharged the next day for subsequent outpatient follow-up.

4. Frequency of Infusion of Transduced Cells
Patients may recieve up to four cycles of transduced PBSC. The cycles will be no more

frequent than every 2 months.

3.4 Risks, Hazards, and Discomforts

This gene transfer protocol would be classified as offering no guarantee of direct benefit to patients enrolled on it, but posing little risk. Information obtained from this pilot study could benefit future patients with Fanconi anemia. None of the over 50 patients who have received bone marrow, peripheral blood or tumor-infiltrating cells transduced by retroviruses have had any adverse events which could be directly attributed to the gene transfer procedure.

Patients will be exposed to the minimal hazards, including bleeding complications and infection, associated with placement of an apheresis catheter each cycle. They will be treated with G-CSF for 7 days every cycle and may experience bone pain, fever, or rash, and less commonly reversible elevation of hepatic enzymes, transient depression of platelet count, and exacerbation of pre-existing inflammatory conditions. During apheresis the patient may experience postural hypotension and paresthesias.

After the end of the treatment portion of the protocol, patients will be requested to participate in further follow-up to evaluate the duration and extent of FACC gene transfer and expression. Follow-up will usually be performed on an outpatient basis and will require needle sticks for blood samples and additional bone marrow aspirates and biopsies. As the patient is involved in a gene therapy trial, a regulatory committee has requested that all patients be followed periodically for detection of side effects from the gene transfer procedure; this request includes that the patient be aware that an autopsy would be requested when the patient dies to evaluate presence and extent of gene transfer.

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

Patients must meet the following criteria within 30 days prior to study entry (Day 0) unless otherwise noted:

4.11 Males or females, age $\geq$ 5 years of age

4.12 Diagnosis of Fanconi anemia, complementation group C, as confirmed by:

4.121 Diepoxybutane or mitomycin C testing

and

4.122 DNA analysis indicating FACC mutations

4.13 Adequate baseline organ function as assessed by the following laboratory values within 30 days prior to study entry (day -30 to 0):

- Adequate renal function with estimated creatinine clearance > 50 ml/min. This will be determined by serum creatinine and 24-hour urine creatinine ordered concurrently.
- Adequate liver function with SGOT, SGPT and alkaline phosphatase $\leq$ 5 times the ULN [if transaminases > than the upper limit of

normal (ULN), patients should have a hepatitis B surface antigen (HBsAG) test prior to study entry. Patients may not enter the study if HBsAG is positive.]

- PT and PTT not more than 1.5 times the ULN
- Serum Amylase $\leq$ 1.5 times the ULN
- Bilirubin $\leq$ 2.5 mg/dL
- Triglyceride < 400 mg/dl

4.14 Ability to give informed consent
4.15 Normal cardiac function by history and exam
4.16 Resting transcutaneous oxygen saturation > 90% on room air
4.17 Karnofsky Performance Status $\geq$ 40 (see Appendix IV)

Although there are no blood count criteria for inclusion in this study, preference will be given to patients with significant marrow failure as reflected by anemia, neutropenia, and/or thrombocytopenia. Furthermore, we intend to first enroll adults and older children, to the extent possible, before enrolling young children.

4.2 Exclusion Criteria

Patients who meet any one of the following criteria will be excluded from study entry:

4.20 Patients presenting with acute leukemia or bone marrow aspirate revealing >10% blasts
4.21 Pregnant or lactating females [all patients must practice adequate birth control and females of child-bearing potential must have a negative serum beta-HCG pregnancy test (within Day -7 to Day 0)].
4.22 Acute infection: any acute viral, bacterial, or fungal infection which requires specific therapy. Acute therapy must have been completed within 14 days prior to study treatment.
4.23 Hepatitis-B surface antigen positive patients.
4.24 HIV-infected patients.
4.25 Acute medical problems such as ischemic heart or lung disease that may be considered an unacceptable anesthetic or operative risk.
4.26 Patients with any underlying conditions which would contraindicate therapy with study treatment (or allergies to reagents used in this study).
4.27 Patients < 25 kg weight or < 5 years of age
4.28 Patients who elect bone marrow transplantation (see below, 13.0 Ethical Considerations)

4.3 Concomitant Medication and Treatment

All allowed medications or treatments should be kept to a minimum and recorded. All questions regarding concomitant medications should be referred to the principal investigator.

4.4 Potential Risks and Discomforts Related to Evaluations

Patients will require needle sticks for blood sampling. They will also undergo bone marrow aspiration and biopsy, with the associated risks of infection and bleeding.

5.0 CLINICAL AND LABORATORY EVALUATIONS

Appendix I is the Schedule of Evaluations Table

5.1 Screening and Baseline Evaluations

The following clinical and laboratory evaluations will occur within Study Days -30 to 0 unless otherwise noted. Screening evaluations, or evaluations that determine patient eligibility, are noted. These screening evaluations must be reviewed prior to study treatment.

NOTE: Day 0 and 1 may be the same date or up to 24 hours apart, however Day 1 begins with treatment initiation.

5.11 Objective Signs and Symptoms: include vital signs (blood pressure, pulse, temperature and respirations) weight and height. (Screening and repeat Day 0)

5.12 History and Physical Examination: Screening, repeat targeted physical exam on Day 0

5.13 Karnofsky Performance Status (see Appendix IV) (Screening)

5.14 Chest X-Ray: Normal CXR within the last 30 days unless new symptoms suggest that this be repeated prior to dosing (Screening)

5.15 Electrocardiogram (ECG): ECG must be normal within 30 days unless new symptoms suggest this be repeated prior to dosing (Screening)

5.16 Urinalysis: Routine urinalysis including a 24-hour creatinine clearance (Screening and repeat Day 0)

5.17 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose (Screening and repeat Day 0)

5.18 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen (Screening and repeat Day 0). Bone marrow aspirate/biopsy.

5.19 Pregnancy Test: Serum pregnancy test must be negative within 7 days prior to Day 0, or between study days -7 and 0.

5.110 Freezing Specimens: Additional serum, plasma, and blood cells should be separated and frozen at baseline (day -1 to day 1, prior to treatment).

5.111 DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences (Baseline).

5.112 MoMLV Antibody Analysis: (Baseline)

5.2 Interval Evaluations

Evaluations are to be done prior to that day's procedures or treatments.

Schedule notation:

Days 0-7: Begin administration of G-CSF to patient

Days 5,6,7: PBSC harvested
Days 5-10: PBSC transduction *ex vivo* with FACC vector
Days 8,9,10: Transduced cells reinfused
Day 11: Patient discharge

5.21 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature and respirations) weight and height will be done Study Days 1-11.

5.22 Physical Examination: Target physical exam will be done on Study Day 0 and before discharge.

5.23 Karnofsky Performance Status (see Appendix IV) will be done before discharge.

5.24 Urinalysis: Routine urinalysis will be done Study Days 8-10 and upon patient discharge.

5.25 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose will be done qod during Study Days 1-11 and at the time of discharge.

5.26 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen will be done daily during Study Days 1-11 and at the time of discharge.

5.27 Pregnancy Test: Serum pregnancy test will be repeated monthly during the first year.

5.28 Freezing Specimens: Additional serum, plasma, and cells should be separated and frozen at time of discharge.

5.29 DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences at time of discharge.

5.3 Post-Treatment Evaluations

For patients completing each PBSC cycle, the following evaluations will be done one month after the patient is discharged and repeated monthly until the next cycle begins. See section 5.4 if patient has completed the protocol or has completed at least one cycle (and has subsequently discontinued):

5.31 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature, respirations) weight and height.

5.32 Physical examination: Targeted physical exam.

5.33 Karnofsky performance status (see Appendix IV).

5.34 Urinalysis: Routine screening urinalysis and creatinine clearance.

5.35 Serum chemistries: Include uric acid, calcium, phosphorus, magnesium, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose.

5.36 Hematology: Complete blood count (CBC),

differential, platelets, and coagulation tests including PT, PTT, and fibrinogen.

5.37 Pregnancy test: Repeated monthly during the first year.

5.38 Freezing specimens: Additional serum, plasma, and cells should be separated and frozen.

5.39 DNA/RNA analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences.

5.310 **Autopsy Request**: All participants in the study will be informed that an autopsy will be requested to determine the extent of gene transfer into important organs such as marrow, liver, and spleen.

5.4 Post-treatment Evaluations for Patients Completing PBSC cycles

Follow-up evaluations will be done on all patients who have completed at least 1 cycle.

See section 5.3. Evaluations will be similar to patients who are between cycles. Patients will be seen every three months for the first year, every six months for the next year and then yearly. In addition to the evaluations outlined in section 5.3, the following will also be done:

5.41 DNA/RNA analysis: Peripheral blood samples (20cc green top).

5.42 DEB sensitivity test: Peripheral blood sample (20 cc green top).

5.43 Bone marrow aspirate: For DNA/RNA analysis months 3, 6, 9, 12 and then yearly afterwards.

5.5 Considerations/Evaluations Upon Premature Withdrawal

5.51 Discontinued patients (see Section 7.0) who have not received transduced cells will not be followed after they are discharged.

5.52 Patients who are discontinued or withdraw before completing all cycles, but have completed at least 1 cycle (patient was reinfused with transduced cells, see Section 7.0) will be asked to return to the clinic to be evaluated according to Section 5.3 and 5.4.

6.0 TREATMENT MODIFICATION AND DISCONTINUATION ACCORDING TO LEVEL OF TOXICITY

The GTI Table for Grading Severity of Adverse Experience (Appendix II) will be used to achieve consistency in response to drug/treatment toxicities. Toxicities will be graded on a 1 - 4 grading scale. If a toxicity is experienced, the treatment level or dose will be modified (if applicable) as outlined below according the grade of toxicity observed.

6.1 Treatment Modification and General Management of Toxicities

- For any Grades 1-2 toxicity, there will be no dose modification.
- Any Grade 3 toxicity, thought to be related to G-CSF, will necessitate a 50% dosage reduction. If Grade 3 toxicity persists for seven days or

advances to grade 4 toxicity, G-CSF will be discontinued and the patient will not re-start the medication. The patient will continue to have apheresis as if G-CSF were administered for the current cycle only. The patient will receive no further G-CSF, and therefore no further cycles of peripheral stem cell harvest will be attempted under this protocol.

- For Grade 3 toxicity not related to G-CSF, the investigators and sponsor will discuss the various options available. The appropriate action relative to the patient with a Grade 3 toxicity as well as a discussion of enrolling other patients will be addressed. Since no toxicities have been seen in other gene transfer protocols, precedence for the appropriate action is not available and will need to be decided on a case-by-case basis.

6.11 Management of Specific Toxicities

Adverse reactions to apheresis: Reactions are rare, and include anxiety, vasovagal reactions, and cutaneous paresthesias related to citrate-induced hypocalcemia. Sedation, volume repletion, and slowing of the infusion will correct these problems. Bleeding and infection at the line insertion site are potential complications of apheresis catheter placement.

6.2 Symptomatic Therapy for Toxicity

Symptomatic therapy such as analgesics or other helpful therapy may be administered if deemed necessary by the participating investigators. All medications administered will be recorded in the appropriate Case Report Form.

7.0 CRITERIA FOR DISCONTINUATION OF STUDY PATIENTS

7.1 Criteria for Treatment Discontinuation

Participation in this study can be discontinued for any of the following reasons listed below:

- At the Patient's request: Any patient who wishes to withdraw from the study may do so but will be counselled that long-term follow-up for the effects of gene therapy are strongly desired by the medical facility and federal regulatory groups.
- A major, unexpected, or life-threatening toxicity requiring that treatment be discontinued
- Generalized impairment or mental incompetence which would render the patient unable to understand his/her participation in the study.
- If, in the Investigator's medical judgment, further participation would be injurious to the subject's health or well-being.
- Administrative reasons, such as subject non-compliance or a major protocol violation.
- Pregnancy

An explanation will be recorded for any patient that is taken off treatment and the appropriate Case Report Form completed.

7.2 Criteria for Study Discontinuation

Patients should understand that once they have completed (at least one cycle of therapy), they will be asked to be followed long-term. They can stop study treatment at any time (see section 7.1), however they will still be considered on study until the study has been terminated.

The study can be discontinued only with the understanding of the investigators, sponsor, RAC and the FDA.

8.0 ADVERSE EXPERIENCES

Depending on the extent of the adverse experience, adverse events should be reported by each site investigator to their local Institutional Review Board (IRB), Institutional BioSafety Committee (IBC) and to Genetic Therapy, Inc. as specified in Appendix III.

Adverse reactions or deaths requiring immediate reporting should be made by telephone to (301) 402-0764 (available 24 hours a day, recorder after working hours).

The principal investigator will take the responsibility of informing and preparing reports for the Recombinant Advisory Committee (RAC) through the NIH Office of Recombinant DNA Activities (ORDA) and to the FDA.

HHS Guidelines for Research Involving Recombinant DNA Molecules: It is the responsibility of the institution and those associated with it to adhere to the intent of the *Guidelines* as well as to their specifics.

9.0 EVALUATION OF RESPONSE/ENDPOINTS

9.1 Study Endpoints:

(i) The study is designed primarily to determine whether the FACC gene can be transferred to hematopoietic progenitors and whether those cells can be readministered to patients without toxicity.

(ii) Endpoints for clinical effect will be primarily evaluated by quantitation of progenitor growth in the presence of MMC; this should serve as a functional assay of efficacy. Other efficacy endpoints will be improvement in peripheral blood counts and marrow cellularity.

9.2 Response Criteria:

No response- Less than 0.1% of circulating mononuclear cells contain the retroviral vector. No RNA signal from the retroviral vector at any time during the study.

Incomplete response

A. Less than 0.1% of circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the vector at any time during the study.

B. More than 0.1% of circulating mononuclear cells contain the retroviral vector. No RNA signal is detected from the vector at any time during the study.

Partial response- More than 0.1% but less than 2% of circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the vector at any time during the study.

Complete response- More than 2% of circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the vector at any time during the study.

9.3 Required Documentation: Documentation will be by molecular biologic techniques done in the Hematology Branch, NHLBI, as well as blood counts and bone marrow pathology reports from the NIH Clinical Center.

10.0 DOCUMENTATION, RECORD KEEPING, CRFs

The investigator must maintain adequate records so that the conduct of the study can be fully documented and monitored.

Copies of protocols, case report forms (CRFs), test result originals, all product accountability records, correspondence, patient informed consent, and any other documents relevant to the conduct of the study must be kept on file by the investigator for (five) years after all investigational use of product is discontinued and the FDA is so notified or until five years after a Product Licensing Application (PLA/ELA) is approved. Study documents should not be destroyed without prior written agreement between GTI and the study investigator. The Study Monitor should be notified if the investigator wishes to assign the study records to another party, or move them to another location.

The investigator will supply GTI on request with any required background data from all patient records and related documents. This may become necessary if the CRFs are illegible or when errors in data transcription are questionable. For FDA inspections, it is also necessary to have access to complete study patient records, provided that patient confidentially is maintained.

The investigator should obtain a separate release of medical information form to be signed by the study patient in order to facilitate access to the patient's medical records should the patient be hospitalized at an institution with which the study investigator is not associated.

A record must be kept of all patients considered (and screened) for the study and subsequently deemed ineligible. The reason for ineligibility must be recorded.

11.0 DATA COLLECTION AND STUDY MONITORING

11.1 Data Collection

Case Report Forms (CRFs) will be utilized for each patient entered into the study. Study participants will NOT be identified by name on any study documents. Patients will be identified by a patient identification number (PIN). Investigators will need to keep a patient code list accessible.

11.2 Study Monitoring

It is understood that the GTI-designated person responsible for study monitoring of patient data will be permitted to contact and visit the investigator at regular intervals and will be allowed, on request, to review original documents such as consent forms, drug accountability records, case report forms, and pertinent hospital or clinic records for confirmation of study data provided that patient confidentiality is maintained in accord with local (IRB) requirements. It will be the monitor's responsibility to verify the adherence to the protocol and the completeness and accuracy of the data being entered on the CRFs. If

local regulations permit, the study monitor should have access to original laboratory test reports and other patient documents needed to verify data entered on the CRFs. The investigator should cooperate with the assigned study monitor to ensure that all problems are resolved.

12.0 BIOSTATISTICAL CONSIDERATIONS

Summaries for the following parameters will be compiled at six months and one year:

-percentage of cells containing the FACC vector
-sensitivity of cells to MMC; progenitor growth in MMC
-blood counts, bone marrow examination
-safety, adverse effects

13.0 ETHICAL CONSIDERATIONS

The investigator will ensure that the study is conducted in full conformance with DHHS and FDA standards for human research.

13.1 Informed Consent

All study participants must sign an informed consent (see Appendix VII). The investigators will inform all subjects as to the nature, aims, duration, potential hazards, and procedures to be performed during the study and that his or her medical records may be reviewed by the sponsors or the FDA. This protocol must receive approval by the Institutional Review Board of all participating sites prior to implementation. The investigator must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time. The protocol will be discussed in detail with all potentially eligible patients. All revisions of the protocol must be reflected in the consent form and reviewed by the IRB.

13.2 Alternative Therapies

Marrow transplantation from allogeneic donors (matched histocompatible siblings) and from alternative donors (matched unrelated normal volunteers or partially mismatched family members) have been successfully employed in some FA patients. All potential participants will be fully informed of the availability and suitability of these procedures, as well as their associated success rates and hazards. Participation in this protocol should not affect marrow transplantation as a future therapeutic option.

13.3 Assent process

For minors, the consent document will be provided to parents or guardians. The consent form will be the basis for the description of the procedures and research therapies to the child and parents. Because of the wide age range of potential participants, discussion will be tailored to the age and sophistication of the child or adolescent. The assent process will take place with several separated visits to the patient, discussions with the patient alone and with the patient in company of his parents, and sufficient time will be allowed for full consideration of the protocol by the patient and family.

13.4 Patient Confidentiality

All reports and patient samples will be identified only by a coded number to maintain patient confidentiality. All records will be kept confidential to the extent permitted by law. The investigator should keep a separate log of patients' codes, names, and addresses. Documents which identify the patient by name (informed consent) should be kept in strict confidence.

14.0 CRITERIA AND PROCEDURES FOR PROTOCOL MODIFICATION AND STUDY TERMINATION

Modifications which may affect the safety of the study patient, or which may alter the scope of the investigation, the scientific quality of the study, the study design, dosages, duration of therapy, patient assessments (added evaluation that poses potential risk or inconvenience to the patient), number of patients, and patient eligibility criteria, may be made only after appropriate consultation between the sponsor and the investigator.

If the consensus is to revise the current protocol, a formal List of Changes will accompany the amended protocol and these will be submitted to the FDA, the site's IRB, and other committees as required.

Both the sponsor and the investigator reserve the right to terminate the study at any time. If this becomes necessary, appropriate procedures for continuing the long-term follow-up requested by the regulatory agencies will be arranged after review and approval by both parties.

15.0 PUBLICATION OF DATA/PROTECTION OF COMPANY SECRETS

The investigator will agree to inform the sponsor of all study results. Any manuscript, abstract, or presentation will be made available to the sponsor and all participating study investigators for review prior to submission of manuscripts, abstracts, or scientific meetings. This will allow the sponsor to protect proprietary information and to provide comment based on information that may not yet be available to the investigator.

16.0 BIOHAZARD WARNINGS AND PRECAUTIONS

Patients will be treated in standard hospital and clinic rooms. No special patient precautions will be taken.

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PROTOCOL SYNOPSIS

Title: RETROVIRAL GENE TRANSFER OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C GENE TO HEMATOPOIETIC PROGENITORS OF GROUP C PATIENTS

Objective:

- Primary Objectives
  The first objective will be to document the feasibility of transferring the FACC gene to hematopoietic progenitors. We hope to mark cells capable of long-term hematopoietic reconstitution.
- Secondary Objectives
  Delivery of the FACC gene and subsequent expression of the gene in cells which carry mutant FACC alleles will, in theory, be therapeutic. We would expect gene-corrected stem cells to maintain a selective advantage within the hypoplastic FA marrow microenvironment. Clinically, this should translate into an improvement in peripheral blood counts in cytopenic patients.

Population: Patients with FA as defined by positive DEB or MMC testing and DNA analysis confirming mutations in FACC alleles will be candidates for this protocol.

Sample Size: This will be a pilot study consisting of six patients.

Dosage/Treatment:

1. Treatment will consist of $\leq$ 4 cycles of peripheral blood progenitor (PBSC) transduction. PBSC harvest will be performed on days 5, 6, 7 following priming with G-CSF at a dose of 10μg/kg/day subcutaneously for 7 days.
2. PBSC will be enriched for CD34 surface expression by immunoaffinity column purification (Cell Pro, Inc.).
3. Enriched PBSC will be transduced with the FACC retroviral vector (via supernatant or stromal support).
4. PBSC will be reinfused into the patient.
5. Cycle will be repeated up to 3 more cycles but no more frequently than every 2 months.

Duration: Patients who complete four cycles will be on study for a minimum of six months. They will be followed as outpatients indefinitely after completion of the protocol.

Endpoints:

- Safety Endpoints-The study is designed primarily to determine whether CD34+ hematopoietic progenitors transduced with a normal FACC gene can be safely reinfused into FA(C) patients.
- Efficacy Endpoints-Response criteria will include quantitation of percentage of mononuclear cells which can be transduced with the FACC retrovirus. Secondary criteria will include assessment of progenitor growth in MMC, changes in peripheral blood counts, and bone marrow cellularity.

Investigators: Principal investigators: Johnson Liu, M.D. and Neal Young, M.D.

Retroviral Mediated Transfer of cDNA for Fanconi's Anemia Type C into Hematopoietic Stem Cells of Patients with Fanconi's Anemia

BACKGROUND AND PURPOSE

Fanconi's anemia is an inherited disorder that can produce bone marrow failure. Some patients with Fanconi's anemia have physical defects, usually involving the skeleton or kidneys. The major problem for most patients is aplastic anemia. In aplastic anemia, the blood counts for red blood cells, white blood cells, and platelets are low because the bone marrow fails to produce these cells. Some patients with Fanconi's anemia also can develop leukemia or cancers in other organs.

The only successful permanent treatment for the bone marrow failure caused by Fanconi's anemia is bone marrow transplantation. Bone marrow transplantation has been performed most successfully using sibling donors whose cells are a perfect match with the patient's. Unfortunately, the majority of patients do not have appropriate family donors. A few cases of Fanconi's anemia have also been cured by bone marrow transplantation from donors other than siblings, as for example from individuals with similar genes from other families, but the risks of this procedure are high. In general, there are many serious risks associated with bone marrow transplantation, especially from alternative donors. Death following bone marrow transplantation from a matched sibling may occur in 25-30 percent of patients and the rate may be higher using other donors. Other types of therapy, including male hormones, corticosteroids, and hematopoietic growth factors may temporarily increase blood counts but do not cure the disease. You (your child) will be eligible for this protocol if you (your child) are not pursuing immediate bone marrow transplantation; however, participation in this protocol will not preclude subsequent bone marrow transplantation if it becomes available for you (your child).

Many laboratory studies have suggested that Fanconi's anemia is due to an inherited defect in the ability of cells to repair DNA. Recently, the gene for one of the four types of Fanconi's anemia, type C, has been identified. This is a novel gene, in that it is not similar to other known genes, and its function has not been characterized. It is known that this gene is defective in patients with Fanconi's anemia type C.

GENE THERAPY FOR FANCONI'S ANEMIA

Studies in our own laboratory have suggested that Fanconi's anemia type C may respond to gene therapy. We have placed the normal Fanconi's anemia type C gene into a virus that acts as a gene-carrier and introduced the gene into cells, derived from Fanconi's anemia type C patients. In the laboratory, when we have compared cell lines and bone marrow cells from Fanconi's anemia patients before and after this procedure, we see a return towards normal of cell growth, resistance to the chemical agents

that harm Fanconi's anemia cells, and a more normal appearance of the cells' chromosomes. Because the cells containing the normal Fanconi's anemia type C gene grow better, these cells may have a competitive advantage compared to unaltered Fanconi's anemia type C cells.

The purpose of this research protocol is to test whether we can safely introduce the normal Fanconi's anemia type C gene into CD34 cells of patients with this disease. As described below in more detail, CD34 cells are primitive blood cells that include stem cells. Stem cells are the cells in the bone marrow and blood that give rise to the white cells, platelets and red cells. We will treat blood cells from patients with Fanconi's anemia type C using the viral carrier containing the Fanconi's anemia gene in a test tube and then return these cells to patients. If the cells are genetically altered, we expect to be able to detect the normal gene in blood and bone marrow cells afterwards. We hope that cells that contain the normal Fanconi's anemia gene will grow well in the bone marrow, and that we will be able to detect normal cells in special tissue culture studies. If we are successful, we should also be able to correct the chromosome abnormality. It may even be possible to increase blood counts in patients with Fanconi's anemia using this procedure. However, it should be clear that the major purpose of this protocol is to test the safety of this technique and to determine whether we can transfer the Fanconi's anemia gene successfully. We do not expect that this protocol will decrease the risk of cancer in the other organs of the body.

Potential benefits: We do not know if there will be any direct benefit to you (your child) from participating in this study. The major goal of this study is to determine if the Fanconi's anemia gene can be transferred safely to patients with type C disease. However, there may be future benefits to you (your child) and to other patients with Fanconi's anemia. It is possible that the results of this study will lead to a new form of experimental therapy for Fanconi's anemia. It is also possible that more prolonged courses of gene therapy would be clinically beneficial. You (your child) would be considered for a such a future protocol.

In order to obtain sufficient CD34 cells from patients with Fanconi's anemia, we will treat each patient with a hematopoietic growth factor called granulocyte-colony stimulating factor or G-CSF. G-CSF will be given for one week, at the end of which time blood cells will be removed by a process called apheresis for treatment with the Fanconi's anemia type C gene vector.

PRESTUDY TESTING

Prior to being entered into this study, you (your child) will undergo blood tests, bone marrow aspiration and biopsy, as well as a full history and physical examination to determine the status of your Fanconi's anemia. You (your child) will have

tests of your liver and kidneys to determine that these organs are functioning well. You (your child) will have approximately 100/ml of blood or 1/2 cup removed for chromosome studies, determination of colony-forming cells, and DNA and RNA analysis. You will sign a separate consent form for the bone marrow aspiration and biopsy, which will be done with a local anesthetic on the 2West Hematology ward. Bone marrow aspiration and biopsy have minimal risks, but do include local pain, bleeding, and infection at the puncture site.

COLLECTION OF PERIPHERAL BLOOD STEM CELLS

G-CSF Administration: To increase the number of immature CD34 cells in the blood, the drug G-CSF will be given to you (your child) either as an outpatient in the clinic or as an inpatient at the Clinical Center. This drug stimulates the bone marrow to release CD34 cells into the blood, where they can be more easily collected. Daily under-the-skin injections of G-CSF will be given for 1 week. G-CSF may cause fever, flu-like symptoms, muscle aches, bone pain, rash, redness at the injection site, a decrease in the platelet count, and temporary worsening of skin conditions such as psoriasis and eczema. You (your child) will be treated for any of these symptoms if they occur. They disappear after discontinuation of the drug.

Central Line Placement: On the 4th or 5th day after starting G-CSF, you (your child) will have a special intravenous line called a "central line" inserted into a large vein in your groin, neck, or chest. This will be done using local anesthesia by a line insertion specialist. You will sign a separate consent for this procedure. This line is needed for the collection of CD34 cells from your blood. If your platelet count is very low, we may give you (your child) a platelet transfusion before inserting this line. You (your child) may have mild pain and bruising at the insertion site. Rarely, infections or more serious bleeding develop at the insertion site, which can be treated by removal of the line, antibiotics, or pressure to the site. It will be removed on day 7, after completion of CD34 cell collection.

Apheresis: On days 5, 6, and 7 after beginning G-CSF, you (your child) will undergo a procedure called apheresis to collect the CD34 cells in your blood. Your circulation will be connected to an apheresis machine through one opening in your central line, and blood will slowly flow through the machine for 1-3 hours. The machine separates out and saves a fraction of your blood containing CD34 cells, while continuously returning red blood cells, platelets, and plasma to you (your child) through the second opening in your central line. After the apheresis ends, the central line will be removed. Apheresis has very few side effects, but some patients experience light-headedness, nausea, or tingling in the fingers and toes. These symptoms can be corrected by slowing down the flow through the machine. The G-CSF will be discontinued on day 7. Repeated apheresis can make patients anemic; there is a very small possibility that you (your

child) could require blood transfusion as a result of this protocol.

Stem cell purification: The cells collected by apheresis will be purified after harvest in the laboratory by a procedure called CD34 selection. The collected cells will be exposed to an anti-CD34 antibody (protein) that sticks to the surface of immature or CD34 cells. The cells will then be purified by a special column that separates the immature cells coated with antibody from more mature cells. This antibody and column procedure are experimental, but they have been used safely to purify CD34 cells from the bone marrow and blood of many patients (over 50) without any known side effects. The cells to which the CD34 antibody bind include hematopoietic stem cells and progenitor cells.

GENE TRANSFER PROCEDURE

Purified CD34 cells will be treated in the laboratory for three days in the presence of a virus that has been engineered to contain the Fanconi's anemia type C gene. Many studies have demonstrated that the gene contained in this viral carrier can be transferred to the chromosomes of host cells. Once the Fanconi's anemia gene is in the host cell, the cell will pass the gene to its daughter cells. The viral carrier or vector is derived from a mouse virus which has been altered specifically so as to be unable to reproduce itself. No side effects have been seen in over 50 patients who have received cells treated with this type of virus vector, although the vector-Fanconi's anemia gene combination has never before been given to patients.

This is a very new procedure, and there may be risks that have not yet been discovered. Theoretically, there may be an increased risk of cancer in cells treated with vectors, but, this risk is minimized by making sure that the vector used can not reproduce itself once it is inside patient cells. Animals and patients receiving cells treated with this type of vector have not developed new cancers attributable to the vector.

Your cells will be grown in several growth factors called IL-3, IL-6 and SCF during vector exposure in the laboratory. These factors are necessary to keep the CD34 cells alive and to increase the chance that the cells will take up the vector. The growth factors are experimental drugs and have been given to patients. Your cells will be washed thoroughly before giving them back to you (your child). We have not detected growth factors on the cells nor in the solution after this washing.

Stem cell reinfusion: After 3-4 days in culture with vector and growth factors, the cells will be collected, washed and given back to you (your child) intravenously. You (your child) will receive three daily infusions of cells on days 8, 9, 10 after beginning G-CSF. Each infusion will take 10-30 minutes, and you (your child) will be monitored closely for side effects during and after the infusions. We do not expect any side effects, but

an allergic reaction is theoretically possible; you (your child) would of course be treated for any reaction that occurred. It is possible that the cells could become infected with bacteria or fungus during the 3 day growth period in the laboratory. If there is any evidence for infection of the cells with unwanted agents, the cells will not be returned to you (your child), but there is a remote chance that you (your child) could acquire a bacterial or fungal infection from the reinfused cells.

Although we do not believe that introduction of the mouse virus vector containing the Fanconi's anemia gene or the growth of your cells in tissue culture should be harmful, we can not be absolutely certain that no risks apply, especially to Fanconi's anemia patients. In Fanconi's anemia, there is a predisposition to leukemia and other cancers, and it is theoretically possible that these treatments might encourage the growth of leukemia cells.

You (your child) will receive an infusion of cells three days after the end of your G-CSF treatment.

OVERALL TREATMENT PLAN

We will repeat this whole treatment procedure up to four times, with at least two months between each treatment. In other words, each patient will be treated with one week of G-CSF, cells will be removed by apheresis for gene therapy, and introduced back into the patient four days later. Two to three months later, this procedure will be repeated, up to a total of four treatments.

FOLLOW-UP

We will perform a number of blood tests to determine whether the normal Fanconi's anemia gene is present in your blood and bone marrow cells and whether it is affecting the way these cells grow in tissue culture. We will also perform tests to make sure the gene transfer procedure is safe. The amount of blood drawn will be approximately four tablespoons every month. We will also need to repeat bone marrow examinations at the time of each gene therapy. Because we need to ensure that the gene transfer procedure does not cause any harmful effects in the bone marrow we will exclude from the protocol patients who refuse these procedures.

Since this study involves a very new approach to patients with Fanconi's anemia, it will be important to maintain contact with you (your child) for the rest of your life so that we can monitor your overall progress and monitor for any possible long term side effects. If the Fanconi's gene is successfully transferred into stem cells, your body might contain the cells carrying the transferred gene for life. We would continue to see you (your child) in our clinic yearly for at least five years. In the event of your death, we would request an autopsy for the purpose

of determining whether the gene has been transferred into important organs, such as the liver or spleen.

It is important to note that participation in this study will in no way alter any of the standard care you (your child) may be receiving. There will be no charge to you (your child) or to your insurance for any of the tests, procedures or hospital stays associated with this study. You will continue to be responsible for the cost of other medical care. You (your child) may withdraw from this study at any time, and we would continue to follow you (your child) as described above without prejudice. You (your child) are then free to explore any other treatment options, standard or experimental.

Potential Risks: The specific risks associated with each component of this protocol were described above. This protocol should be considered as offering no guarantee of direct benefit to you (your child), but posing some risk. In any new form of therapy, there may be risks which are unknown or not anticipated. Since the vector does not replicate inside the body, we do not anticipate that it can alter your sperm/ova, and therefore we do not anticipate that this treatment could adversely affect your offspring. Participation in this study will not lower the risk of passing Fanconi's anemia to your offspring.

Women of child-bearing potential are advised to use an effective method of birth control until the safety of this experimental therapy has been fully established. Men are advised to use barrier-protection while participating in this study. Females must have had a negative pregnancy test within 14 days prior to dosing. If you should become pregnant while on this study you must inform the investigator immediately.

CURRICULUM VITAE

Name: Johnson M. Liu

Date, Place of Birth: February 2, 1963; Worcester, Massachusetts

Education: B.S., Univ. of Michigan 1984
M.D., Univ. of Michigan 1986

Brief Chronology of Employment:

1993-	Senior Research Investigator, Hematology Branch, NHLBI, Bethesda, MD
1989-1993	Medical Staff Fellow Clinical Hematology Branch, NHLBI, Bethesda, MD
1988-1989	Clinical Associate, NHLBI, Bethesda, MD
1987-1988	Resident, Dept. of Medicine, MUSC, Charleston, SC
1986-1987	Intern, Dept. of Medicine, MUSC, Charleston, SC

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CURRICULUM VITAE

Name: Neal Stuart Young

Date, Place of Birth: April 13, 1947; New York City, New York

Education: A.B., cum laude, Harvard College, 1967
M.D., Johns Hopkins School of Medicine, 1971

Brief Chronology of Employment:

1993-	Chief, Hematology Branch and Acting Chief, Clinical Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland
1989-	Head, Clinical Services, Hematology Branch
1983-1993	Chief, Cell Biology Section, Clinical Hematology Branch
1981-1983	Senior Investigator, Clinical Hematology Branch
1976-1981	Visiting Expert, Clinical Hematology Branch
1975-1976	Clinical Fellow, Hematology-Oncology Division, Barnes Hospital, Washington University, St. Louis, Missouri
1973-1975	Research Associate, Laboratory of Chemical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland
1971-1973	Medical Intern and Assistant Resident, Massachusetts General Hospital, Boston, Massachusetts

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Young, N.S. and Alter, B.F.: Aplastic Anemia, Acquired and Inherited. W.B. Saunders, Philadelphia, 1993.

HUMAN GENE TRANSFER PROTOCOL

9406-079 Roth, Jack A.; MD Anderson Cancer Center, Houston, Texas; *Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin.*

Date of RAC Approval: 6/10/1994

Patient Eligibility: Patients must have histologic proof of non-small cell lung cancer. Patients must be either unresectable, unable to receive primary external beam radiation therapy, or have failed primary external beam radiation therapy, or have failed chemotherapy.

Patients must have either an endobronchial tumor accessible by the bronchoscope. with some clinical evidence of bronchial obstruction, advanced local-regional cancer which is unresectable, or malignant pleural effusion.

All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).

A tumor biopsy must show a p53 mutation by single-strand conformation analysis.

Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative.

Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of $>2{,}000/mm^3$ and platelet count of $100{,}000/mm^3$), adequate liver function (bilirubin ≤ 1.5 mg/dl), and adequate renal function (creatinine < 1.5 mg/dl).

Treatment Plan: The study will be done in two phases for each patient. It is not known what toxicities if any will be caused by the adenovirus. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive one intratumor or intrapleural injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU). Following completion of the first vector-alone injection, a second phase will evaluate Ad5CMV-p53 and cisplatin administered concurrently. Two weeks later the patient will receive one intratumor or intrapleural injection of Ad5CMV-p53 at the same dose as the first injection with concurrent cisplatin at 30 mg/m^2 with 2 additional doses of cisplatin on days 2 and 3. Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitation imposed by production of the adenovirus). The adenovirus dose will increase in one $\log_{10}$ increments for each group. Patients entered at a given dose level will not be eligible for dose escalation. Dose escalation will proceed separately for the intratumor injection group and the intrapleural injection group. The dose of cisplatin will remain constant.

Patient Evaluation: Patients will have a CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray prior to each course of therapy. Serum will be collected pre- and post-treatment for analysis of antibodies to adenovirus proteins.

The endobronchial tumors will be photographed bronchoscopically at the beginning of each course. Tumor measurements are to be recorded before each course for all measurable tumors.

Fine needle aspirates or core biopsies will be obtained of accessible local tumor. Cells in the cytospins of pleural effusions will be obtained pre- and post-treatment when possible. For endobronchial tumors bronchoscopic tumor and normal bronchial epithelial biopsies will be obtained prior to the beginning of each course. Tissue will be fixed immediately in 4% paraformaldehyde and 0.5% gluteraldehyde at 4°C. This will permit extraction of DNA and RNA and permit in situ hybridization.

Biopsies will be analyzed for uptake of the transduced gene by DNA PCR and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction and by in situ hybridization.

All patients will be evaluable for response and toxicity following one course of therapy.

Statistical Considerations: Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitations imposed by production of the adenovirus). A maximum of 21 patients will be entered in each phase of the study.

Objectives:

1. To determine the maximum tolerated dose of the wild-type p53 adenovirus vector given with and without cisplatin in patients with refractory non-small cell lung cancer.
2. To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.
3. To document observed antitumor activity of this treatment approach.

Protocol Title: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin*

Principal Investigator: Jack A. Roth, M.D.

The purpose of this protocol is to study a way of stopping cancer causing genes from functioning in human lung cancer cells in patients. One gene that is commonly altered in human lung cancer is the p53 tumor suppressor gene. A mutation or deletion of both copies of this gene causes the cell to become cancerous.

This protocol uses a gene therapy technique to replace the normal function of the p53 gene. The normal p53 gene will be introduced into lung cancer cells. This normal gene will overcome the function of the mutant gene and also replace the normal p53 function.

A novel feature of this protocol is that the gene will be introduced into human cancer cells in the patient by an adenovirus. A special adenovirus that is harmless to patients and rendered incapable of dividing is used. The adenovirus is taken up by the cancer cell but is not integrated into the cancer cell's DNA. The newly inserted gene is now expressed for a short period of time. This burst of expression by the p53 gene renders the cancer cell incapable of growing and dividing and the cell initiates a program that leads to its death. A common chemotherapy agent (drug) called cisplatin will be given along with the adenovirus. The adenovirus potentiates the cancer killing ability of this drug. The normal p53 gene is not harmful to normal cells. The adenovirus will be injected directly into the lung cancer.

Patients with lung cancer who are unable to have surgery, radiation therapy, or chemotherapy may participate. The tumor will be tested to determine if it has one or the other genetic abnormality. The adenovirus capable of correcting the specific gene abnormality will be directly injected into the tumor. One injection will be of the adenovirus alone and the second injection will be of the adenovirus followed by intravenous administration of the cisplatin. Following this the growth of the tumor will be measured. The tumor will also be studied to determine if the tumor cells are taking up the adenovirus.

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Protocol Title: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin*

Abstract

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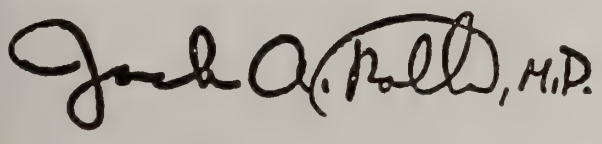
Appendix A: Evaluation Before and During Therapy
Appendix B: Zubrod Scale of Performance Status
Appendix C: Toxicity Criteria
Appendix D: Figures/Figure Legends
Appendix E: Biosafety Precautions
Appendix F: Informed Consent

STUDY CHAIRMAN:



Jack A. Roth, M.D.
Department of Thoracic and Cardiovascular Surgery

STUDY COORDINATORS: Departments of Thoracic and Cardiovascular Surgery, Thoracic, Head and Neck Medical Oncology, and Radiation Therapy.

M.J. McMurtrey, M.D.

J. Nesbitt, M.D.

J.B. Putnam, Jr., M.D.

G.L. Walsh, M.D.

F.D. Martin, R.N.

D. Schrump, M.D.

W.K. Hong, M.D.
Dept of Thoracic, Head & Neck Oncology

R. Komaki, M.D.
Department of Radiotherapy

Wei-Wei Zhang, M.D.

Frank Fossella, M.D.
Department of Thoracic, Head & Neck Oncology

Dong Shin, M.D.
Department of Thoracic, Head & Neck Oncology

Cesar H. Carrasco, M.D.
Department of Diagnostic Imaging

PROTOCOL THS 94-002
JANUARY 27, 1994

STUDY COORDINATORS: Departments of Thoracic and Cardiovascular Surgery, Thoracic, Head and Neck Medical Oncology, and Radiation Therapy.

Michael J. Imperiale, Ph.D.
Dept. of Microbiology & Immunology
University of Michigan Medical School

PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title)

Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin

Study Chairman: Jack A. Roth, M.D.

Patient Eligibility:

Patients must have histologic proof of non-small cell lung cancer. Patients must be either unresectable, unable to receive primary external beam radiation therapy, or have failed primary external beam radiation therapy, or have failed chemotherapy.

Patients must have either an endobronchial tumor accessible by the bronchoscope. with some clinical evidence of bronchial obstruction, advanced local-regional cancer which is unresectable, or malignant pleural effusion.

All patients must have a life expectancy of at least 12 weeks and must have a performance status of ≤ 2 (Zubrod scale, Appendix B).

A tumor biopsy must show a p53 mutation by single-strand conformation analysis.

Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative.

Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of $> 2{,}000/mm^3$ and platelet count of $100{,}000/mm^3$), adequate liver function (bilirubin ≤ 1.5 mg/dl), and adequate renal function (creatinine < 1.5 mg/dl).

Treatment Plan: (Include dose adjustment)

The study will be done in two phases. It is not known what toxicities if any will be caused by the adenovirus. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive one intratumor or intrapleural injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU). Following completion of the first vector-alone group, a second phase will evaluate Ad5CMV-p53 and cisplatin administered concurrently. Patients in the second group will receive one intratumor or intrapleural injection of Ad5CMV-p53 with concurrent cisplatin at 30 mg/m^2 with 2 additional doses of cisplatin on days 2 and 3. Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitation imposed by production of the adenovirus) for each group. The adenovirus dose will increase in one $\log_{10}$ increments for each group. Patients entered at a given dose level will not be eligible for dose escalation. Dose escalation will proceed separately for the intratumor injection group and the intrapleural injection group. The dose of cisplatin will remain constant.

Pretreatment and Treatment Evaluation:

Patients will have a CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray prior to each course of therapy. Serum will be collected pre- and post-treatment for analysis of antibodies to adenovirus proteins.

The endobronchial tumors will be photographed bronchoscopically at the beginning of each course. Tumor measurements are to be recorded before each course for all measurable tumors.

Fine needle aspirates or core biopsies will be obtained of accessible local tumor. Cells in the cytospins of pleural effusions will be obtained pre- and post-treatment when possible. For endobronchial tumors bronchoscopic tumor and normal bronchial epithelial biopsies will be obtained prior to the beginning of each course. Tissue will be fixed immediately in 4% paraformaldehyde and 0.5% gluteraldehyde at 4°C. This will permit extraction of DNA and RNA and permit in situ hybridization.

Biopsies will be analyzed for incorporation of the transduced gene into the host genomic DNA and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction and by in situ hybridization.

All patients will be evaluable for response and toxicity following one course of therapy.

Miscellaneous Information:

Not applicable

Statistical Considerations:

Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitations imposed by production of the adenovirus). A maximum of 21 patients will be entered in the intratumor and the intrapleural groups, respectively for each of the treatment groups (Ad5CMV-p53 alone and Ad5CMV-p53 plus cisplatin).

Objectives:

1. To determine the maximum tolerated dose of the wild-type p53 adenovirus vector given with and without cisplatin in patients with refractory non-small cell lung cancer.
2. To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.
3. To document observed antitumor activity of this treatment approach.

Clinical Protocol Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype p53 and Cisplatin"

1.0 OBJECTIVES

1.1 To determine the maximum tolerated dose of the wild-type p53 adenovirus vector given with and without cisplatin in patients with refractory non-small cell lung cancer.

1.2 To determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment approach.

1.3 To document observed antitumor activity of this treatment approach.

2.0 BACKGROUND AND RATIONALE

2.1 Molecular events in NSCLC

Lung cancer remains the leading cause of cancer deaths in the United States where it kills more than 140,000 people annually. Recently, age-adjusted mortality from lung cancer has surpassed that from breast cancer in women. Although implementation of smoking-reduction programs has decreased the prevalence of smoking, lung cancer mortality rates will remain high well into the 21st century[1]. Unfortunately, all current treatment modalities, including radiation therapy, surgery, and chemotherapy, have limited effectiveness. The rational development of new therapies for lung cancer will depend on an understanding of the biology of lung cancer at the molecular level. Research in our laboratory has identified critical molecular events leading to NSCLC development and progression. The goal of this research is to directly modify the cancer cell to eliminate the expression of gene products which are responsible for the maintenance or progression of the malignant phenotype or to restore in normal form deleted or mutated gene products that suppress the characteristics of the malignant phenotype.

The purpose of this protocol is to investigate molecular mechanisms that may influence the growth and progression of human lung cancer; our goal is development of therapeutic agents specifically targeted at the molecular level. The most common lung cancer histologies (80%) are grouped under the term non-small-cell lung cancer (NSCLC) and include squamous, adenocarcinoma, and large-cell undifferentiated. Many of the current data on the molecular biology of lung cancer come from the study of the more uncommon small-cell lung cancer (SCLC). SCLC can be distinguished from NSCLC by the neuroendocrine features of the cells; SCLC is very responsive to chemotherapy but recurs rapidly after treatment. NSCLC also may serve as a model for other carcinogen-induced malignancies. The approaches and observations developed in this study may be applicable to other types of epithelial cancers.

Abundant evidence has accumulated that the process of malignant transformation is mediated by a genetic paradigm[2]. The major lesions detected in cancer cells occur in dominant oncogenes and tumor suppressor genes. Dominant oncogenes have alterations in a class of genes called proto-oncogenes, which participate in critical normal cell functions, including signal transduction and transcription. Primary modifications in the dominant oncogenes that confer the ability to transform include point mutations, translocations, rearrangements, and amplification. Tumor suppressor genes appear to require homozygous loss of function, by mutation, deletion, or a combination of these for transformation to occur. Some tumor suppressor genes appear to play a role in the governance of proliferation by regulation of transcription. It is possible that modification of the expression of dominant and tumor suppressor oncogenes may influence certain characteristics of cells that contribute to the malignant phenotype.

Despite increasing knowledge of the mechanisms involved in oncogene-mediated transformation,

little progress has occurred in developing therapeutic strategies that specifically target oncogenes and their products. Initially, research in this area was focused on dominant oncogenes, as these were the first to be characterized. DNA-mediated gene transfer studies showed acquisition of the malignant phenotype by normal cells following the transfer of DNA from malignant human tumors. Activated oncogenes of the *ras* family were identified by this technique with transfection of human DNA into mouse NIH 3T3 cells. More recently a class of tumor suppressor genes have been identified. Mutation or deletion of both copies of a tumor suppressor gene is required to eliminate its function and cause the cell acquire characteristics of the malignant phenotype.

Tumor Suppressor Gene Mutations in Lung Cancer

The *p53* gene is the most frequently mutated gene yet identified in human cancers. It is mutated in over 50% of human NSCLC[3]. The *p53* gene encodes a 375-amino-acid phosphoprotein that can form complexes with viral proteins such as large-T antigen and E1B[4]. Missense mutations are common for the *p53* gene and are essential for the transforming ability of the oncogene. The wildtype *p53* gene may directly suppress uncontrolled cell growth or indirectly activate genes that suppress this growth. Thus, absence or inactivation of wildtype *p53* may contribute to transformation. However, some studies indicate that the presence of mutant *p53* may be necessary for full expression of the transforming potential of the gene. Mutations of *p53* are common in a wide spectrum of tumors[5-8]; they occur in both NSCLC and SCLC cell lines and fresh tumors[8,9].

An option for specific targeting of tumor suppressor genes is replacement of a deleted or mutated tumor suppressor gene. Progress in the understanding of the critical genes involved in tumor development and in technology for altering gene expression logically led to our studies of techniques for achieving these options.

The working hypothesis that we developed is that reversal of a single altered genetic event in the cancer cell can potentially reverse critical features of the malignant phenotype of that cell. This finding has important therapeutic implications. Cancer cells have multiple genetic alterations. Therapy directed toward oncogenes will be practical only if therapeutic effects occur with targeting of one or two genes. It is unlikely that any therapy targeting oncogenes or their products will be absolutely specific for cancer cells. If other genes can compensate for loss of normal function by a specific oncogene mediated by an antisense construct, the harmful effects of the therapy will be reduced. Studies from our laboratory indicate that reversal of a single genetic alteration has profound effects on the growth and tumorigenicity of lung cancer cells[10,11]. Additional support for this concept comes from a recent study by Soriano and coworkers[12] in which transgenic mice were created that lacked a functional *c-src* proto-oncogene. The resulting developmental defect in the mice was osteopetrosis. The ubiquity of *c-src*, its high degree of conservation among species, and its role in mitosis suggest that inactivation would be lethal, but this was not the case; viable mice were recovered. A possible explanation is that other closely related nonreceptor tyrosine kinases such as *yes* and *fyn* can compensate for loss of *c-src*. Introduction of a single copy of a wildtype tumor suppressor gene into normal cells would be unlikely to have adverse effects if it occurred during therapy directed at replacing inactivated tumor suppressor genes in cancer cells.

Data on transfection of an antisense K-*ras* expression vector indicated that inhibition of expression of a single oncogene reduced the growth rate of cancer cells and tumorigenicity in nu/nu mice. However, transfected cells retained viability, as did cells with no endogenous K-*ras* mutation that were also transfected with the construct. The wt*p53* appears dominant over the mutant gene and will select against proliferation when transfected into cells with the mutant gene[11,13]. Normal expression of the transfected wt*p53* does not affect the growth of cells with endogenous wt*p53*. Thus, such constructs might be taken up by normal cells without adverse effects. This protocol will study regional delivery of wt*p53* to lung cancer cells in patients with unresectable obstructing endobronchial cancers, unresectable local cancers, and malignant pleural effusions not treatable by conventional therapies. The efficiency of delivery and gene expression will be evaluated both in lung cancer cells and in normal cells in vivo. This is of importance for the design of constructs that may be useful therapeutically. The effects of these constructs on clinical progression of the cancer will be studied.

These approaches may lead to cancer therapy based on direct alteration of gene expression in cancer cells. Current therapy relies on attempts to kill or remove the last cancer cell. However, tumor cell dormancy is an established phenomenon making effective killing highly unlikely. Although inhibition of expression of some oncogenes may be lethal to the cancer cell, in some cases cell replication will slow or cease, thus rendering these cancers clinically dormant. Even if absolute specificity is not achieved, single oncogenes or tumor suppressor genes may still be important targets, because it is likely that adverse effects to normal cells will be minimal.

Programmed cell death, also known as apoptosis, shows a characteristic pattern of DNA fragmentation resulting from cleavage of nuclear DNA and is considered to be a selective process of physiologic cell deletion. It has recently been reported that the wt-*p53* gene is involved in mediating programmed cell death of some types of tumor cells including human lung cancer cells[14-16]. The loss of *p53* function may contribute to resistance to a variety of chemotherapeutic agents[17]. Our studies showed that H358a cells, which have a homozygous *p53* deletion, were resistant to cisplatin *in vitro*. Recombinant adenovirus-mediated wt-*p53* gene transfer into monolayer cultures or multicellular tumor spheroids of H358a resulted in a marked increase in cellular sensitivity to cisplatin. The dying *p53*-transduced cells showed apoptosis with specific DNA fragmentation. Direct injection of *p53* adenovirus into H358a tumors subcutaneously implanted in *nu/nu* mice, followed by intraperitoneal administration of cisplatin, induces massive apoptotic destruction of established tumors. These results support the use of this strategy in a clinical trial.

2.2 Natural history of locally unresectable NSCLC

Patients with NSCLC will die of their cancer in 86% of cases. Regional delivery of gene constructs to areas at risk for development of cancer has important implications for both prevention and therapy. Failure of therapy at the primary tumor site is a significant problem[18,19]. Of the 161,000 patients newly diagnosed with lung cancer in 1991, 45,080 will undergo surgical resection. Local recurrence as the first site of failure will occur in 9,000 of those patients. Of the remaining patients, 52% will have localized tumors. However, 38% of these patients will have local failures following radiation therapy (22,900). Thus, 31,900 patients per year could benefit from improved local-regional therapy. Patients with unresectable obstructing NSCLC that is resistant to radiation therapy or who have coexisting metastases have a median survival of 6 months or less[20]. The Department of Thoracic and Cardiovascular Surgery at the University of Texas M. D. Anderson Cancer Center has extensive experience in the treatment of lung cancer. Over 1200 patients with lung cancer are seen yearly and over 200 of these patients undergo resection.

2.2.1 Measure of disease activity

The goal of this therapy is to halt or reverse the manifestations of the disease. The efficacy of therapy in this group of patients will be measured by determining length of patient survival, length of time the affected lobe of the lung remains aerated, reduction in measurable endobronchial tumor or measurable tumor mass, or reduction in the volume of pleural effusion with clearing of malignant cells. There is no curative therapy for this stage of disease and thus the outcome is predictable enough to allow for an assessment of the results of gene therapy. The measurements that will be used are described in Section 7.0.

2.2.2 Anticipated effect of protocol treatment

It is anticipated that the administration of the adenovirus constructs with cisplatin will decrease the rate of proliferation of these cells. This would increase the length of time the affected lung would remain expanded, prevent regrowth of the endobronchial tumor, reduce the growth rate or cause regression of locally extensive cancers and pleural effusions and therefore prolong the patient's survival and relieve symptoms.

2.2.3 Alternative therapies

Patients with unresectable endobronchial tumor recurrence that is partially or completely

obstructing the airway and that have failed or are unable to receive external beam radiotherapy will be considered for this protocol. Existing therapies for this condition offer only short-term palliation. Most patients have recurred despite external beam radiotherapy. It may be possible to insert a brachytherapy catheter and administer additional radiotherapy. Patients receiving this treatment have a median survival of 6 months[20]. Patients failing brachytherapy would also be eligible to receive gene therapy. Tumor can be removed from the airway with the laser or biopsy forceps. This can be done in conjunction with injection of the adenovirus construct thus decreasing the volume that must be injected. Patients with unresectable local-regional tumors who have failed surgery or radiation therapy have a poor prognosis. Chemotherapy is only palliative and the median survival is less than 6 months. Patients with malignant pleural effusions are not curable and are usually treated symptomatically with sclerosis. The administration of the adenovirus constructs would not preclude the patient from receiving other palliative therapy if the tumor progresses.

2.3 Structure and characteristics of the biological system

2.3.1 Restoration of expression of wt*p53* gene product

2.3.1.1 Preliminary studies with plasmid DNA

The *p53* gene is the most commonly altered gene yet described in human cancers. To study this gene, a cell culture model system of cell lines varying in *p53* expression was established. The H322a lung adenocarcinoma cell line expresses the mutant *p53* protein as shown by the presence of high levels of endogenous *p53* mRNA and phosphorylated protein. We showed that the H322a cell line has a G:T transversion at codon 248 (Arg to Leu) with absence of the wildtype allele. The H358a cell line has a homozygous *p53* deletion. The H460a and H226b cell lines are homozygous for the wildtype *p53*. Expression vectors for sense (S-*p53*) and antisense *p53* (AS-*p53*) cDNA with a β-actin promoter were constructed to study the effect of wt*p53* expressed in lung cancer cells with mutant or deleted *p53* and the effects of reducing wildtype and mutant *p53* expression[11].

Stable transfectants of *p53* mutant cells (H322a) or deleted *p53* (H358) expressing S-*p53* could not be rescued. Failure to isolate colonies expressing sense *p53* RNA in cells with homozygous mutant or deleted alleles shows that wt*p53* can suppress transformation in cancer cells expressing a mutant *p53* or having a homozygous *p53* deletion.

In general, transfection with antisense *p53* (AS-*p53*) reduced colony formation (10-fold) by cells with endogenous mutant *p53*. This indicates that expression of mutant *p53* contributes to the transformed phenotype. As expected, cells with wt*p53* (H226b) showed increased tumorigenicity when transfected with AS-*p53*. The H226b cells expressing AS-*p53* grow significantly more rapidly in nu/nu mice than the cells transfected with the control plasmid. This indicates that elimination of the wt*p53* gene product enhances features of the malignant phenotype.

Our studies showed that wt*p53* is dominant and can suppress the malignant phenotype in cells with mutant or deleted *p53*. The presence of the mutant *p53* confers transforming potential to the gene product, which can be suppressed by AS-*p53*. Thus, in cancer cells both the absence of wt*p53* and the presence of certain *p53* mutations may enhance the malignant phenotype.

2.3.1.2 Generation of recombinant p53 adenovirus.

The p53 expression cassette (Figure 1, Appendix D), which contains human cytomegalovirus (CMV) promoter[21], wild-type p53 cDNA, and SV40 early polyadenylation signal, was inserted between the Xba I and Cla I sites of pXCJL.1, a plasmid kindly provided by Dr. Frank L. Graham of McMaster University, Hamilton, Ontario, Canada. The p53 shuttle vector (pEC53) and the recombinant plasmid pJM17[22] were cotransfected into 293 cells[23] by liposome-mediated transfection with DOTAP (Boehringer Mannheim Corp., Indianapolis, IN)[24]. The transfected cells were maintained in medium until the onset of the cytopathic effect. Identification of newly generated p53 recombinant adenoviruses (Ad5CMV-p53) with PCR analysis of the DNA samples prepared from the cell culture supernatants was described elsewhere[24]. The wild-type sequence of the p53 cDNA in the Ad5CMV-p53 virus was confirmed by dideoxy DNA sequencing on the CsCl-gradient-purified viral DNA. The control virus Ad5/RSV/GL2, generated in a similar manner, has a structure similar to that of Ad5CMV-p53 except a Rous sarcoma viral promoter and luciferase cDNA were used in its expression cassette. The recombinant adenovirus that carries a *E. coli* b-galactosidase gene (LacZ), Ad5CMV-LacZ, also has a structure similar to that of Ad5CMV-p53, and was kindly provided by Dr. Frank L. Graham. Structural analysis of the vectors is shown in Fig. 2, Appendix D.

2.3.1.3 Viral stock, titer, and infection. Individual clones of the Ad5CMV-p53, Ad5/RSV/GL2, and Ad5CMV-LacZ viruses were obtained by plaque-purification according to the method of Graham and Prevec[25]. Single viral clones were propagated in 293 cells. The culture medium of the 293 cells showing the completed cytopathic effect was collected and centrifuged at 1000 x *g* for 10 min. The pooled supernatants were aliquoted and stored at -20°C as viral stocks. The viral titers were determined by plaque assays[25]. Infections of the cell lines were carried out by addition of the viral solutions (0.5 ml per 60-mm dish) to cell monolayers and incubation at room temperature for 30 min with brief agitation every 5 min. This was followed by the addition of culture medium and the return of the infected cells to the 37°C incubator.

2.3.1.4 Preclinical studies

Expression of exogenous p53 in human lung cancer cells. To obtain a high level expression of p53, the human CMV promoter[21] was used to drive the p53 cDNA carried by Ad5CMV-p53. As shown by immunostaining and Western blot in Figures 3 and 4 (Appendix D), a high level of expression of exogenous p53 was achieved in the H358 cells that were infected by Ad5CMV-p53 at an MOI of 30 PFU/cell. When H322 or H460 cells were infected at the same MOI, the level of expression of the exogenous p53 gene was three times higher than that of the endogenous mutated protein in H322 and 14 times higher than that of the endogenous wild-type protein in H460 cells. The time course of the expression of the exogenous p53 after a single infection of 10 PFU/cell was studied in H358 cells. The protein expression peaked at postinfection day 3, sharply decreased after day 5, and lasted for at least 15 days (Fig. 5, Appendix D). PCR analysis on the DNA samples prepared from the Ad5CMV-p53-treated H358 cells failed to detect the viral DNA after postinfection day 15 (data not shown). The decrease in expression of the exogenous p53 probably resulted from the cellular attenuation on the CMV promoter or degradation of the viral DNA in the treated cell population[26]. This is a critical point with respect to safety of the vector. Transient *p53* expression is sufficient for mediating apoptosis. However, normal cells taking up the vector will express the exogenous *p53* for only a short time.

Effect of exogenous p53 on lung cancer cell growth. Three human NSCLC cell

lines were chosen for this study: cell line H358, which has a homozygous deletion of the p53 gene, cell line H322, which has a point mutation of the p53 gene at codon 248 (G to T), and cell line H460, which has a wild-type p53 gene. The cells were treated with Ad5CMV-p53 and Ad5/RSV/GL2 at 10 PFU/cell. Triplicate sets of the viral-infected and mock-infected cells were counted every day for 6 days. Growth rates of the Ad5CMV-p53-infected H358 and H322 cells were inhibited 79% and 72%, respectively, compared to that of the mock-infected cells (Fig. 6. A and B, Appendix D). The growth rate of the Ad5CMV-p53-treated H460 cells was inhibited by 28% of that of the mock-infected H460 cells (Fig. 6C, Appendix D). Cells infected with the control virus Ad5/RSV/GL2 had growth rates similar to those of the mock-infected cells. When the Ad5CMV-p53-infected cells were harvested at 24 h after infection and replated in cell culture dishes, only 14% of the treated H358 and H322 cell populations reattached and survived, whereas the mock-infected cells had a replating efficiency of more than 95%. This suggested that the viability of the Ad5CMV-p53-infected H358 and H322 cells was greatly reduced.

Inhibition of tumorigenicity mediated by Ad5CMV-p53. To examine whether the Ad5CMV-p53 virus can inhibit tumorigenicity of human NSCLC cells, nude mice were injected s.c. with H358 cells to induce tumor. Each mouse received one injection of $2x10^6$ cells that had been infected with Ad5CMV-p53 or control vector Ad5/RSV/GL2 at 10 PFU/cell for 24 h. H358 cells treated with medium alone were used as mock-infected controls. Tumors, first palpable at postinjection day 14, were induced only by the mock- or control virus-infected cells (Table I); mice that received Ad5CMV-p53-treated cells did not develop tumors (Table I). The tumors at the end of a 6-week period were 4-10 mm in diameter. This study was initiated with five mice per group; one mouse each in the Ad5CMV-p53 or Ad5/RSV/GL2 group failed to complete the study. The early deaths were presumably due to infection, since the mice that received injections were kept in a regular animal room after they were taken out from a barrier area for radiation treatment.

Table I. Effect of Ad5CMV-p53 on tumorigenicity in H358 in nude mice*

Treatment	No. of Tumors/ No. of Mice (%)	Mean Volume (mm^3 ± SD)
Medium	4/5 (80)	37 ± 12
Ad5/RSV/GL2	3/4 (75)	30 ± 14
Ad5CMV-p53	0/4 (0)	—

* The treated H358 cells were injected s.c. at 2 x 10^6 cells/mouse. Tumor sizes were determined at the end of a 6-week period.

The efficacy of Ad5CMV-p53 in inhibiting tumorigenicity was further evaluated in the mouse model of orthotopic human lung cancer[27]. Since H358 and H322 cells did not produce tumors in this model, cell line H226Br was used. This cell line has an origin of squamous lung cancer and metastasized from lung to brain. It has a point mutation (ATC to GTC) at exon 7, codon 254, of the p53 gene and is tumorigenic in mice. The irradiated nude mice were inoculated with 2 x 10^6 H226Br cells/mouse by intratracheal instillation. Three days after inoculation, each of the mice (8-10 mice per group) were treated with 0.1 ml of either Ad5CMV-p53 or Ad5/RSV/GL2 or vehicle (PBS) by intratracheal instillation once a day for two days. The virus dosage used was 5 x 10^7 PFU/mouse. Tumor formation was evaluated at the end of a 6-week period by dissecting the lung and mediastinum tissues and measuring the tumor size. The procedure is depicted in Figure 7, Appendix D. The representative samples of dissection are demonstrated in Figure 8, Appendix D. The detected

tumors were confirmed by histologic analysis. The data of tumor measurements are summarized in Table II: only 25% of the Ad5CMV-p53-treated mice formed tumors, whereas in the vehicle or Ad5/RSV/GL2 control group, 70-80% of the treated mice formed tumors. The average tumor size of the Ad5CMV-p53 group was significantly smaller than those of the control groups. These results indicate that Ad5CMV-p53 can prevent H226Br from forming tumors in the mouse model of orthotopic human lung cancer.

Table II. Effect of Ad5CMV-p53 on tumorigenicity in H226Br in mouse model of orthotopic human lung cancer[a]

Treatment	No. mice w/Tumor Total Mice (%)	Mean Volume (mm^3 ± SD)
Vehicle	7/10 (70)	30 ± 8.4
Ad5/RSV/GL2	8/10 (80)	25 ± 6.9
Ad5CMV-p53	2/8 (25)	8 ± 3.3[b]

[a] Mice were inoculated with 2 x 10^6 H226Br cells/mouse intratracheally. On the 3rd day post-inoculation, the mice were given either vehicle or viruses (5 x10^7 each in 0.1 ml) intratracheally once a day for 2 days. Tumor formation was evaluated at the end of a 6-week period.

[b] $p < .0.05$ by two-way analysis of variance when compared to the groups receiving vehicle (PBS) or virus control.

Some human lung cancer cells such as H358a are resistant to both p53 and chemotherapy induced apoptosis. In an effort to confirm the feasibility of this combination therapy in human lung cancer, we examined whether Ad-p53 and CDDP given in a sequential combination could induce apoptosis in vivo. Following 3-day direct intratumoral injection of Ad-p53, H358a tumors subcutaneously transplanted in nu/nu mice showed a modest slowing of growth; Ad-p53-injected tumors, however, regressed if CDDP was administered intraperitoneally for 3 days, and the tumor size remained almost consistent for more than 12 days by multiple cycle treatments with Ad-p53 and CDDP (Fig. 8A, Appendix E). Histologic examination revealed a massive destruction of tumoral tissues in the area where Ad-p53 was injected in mice treated with CDDP. In situ staining of programmed cell death demonstrated many apoptotic cells around the dead space (Fig. 8B-E, Appendix E). In contrast, tumors treated with CDDP alone or Ad-p53 alone showed neither empty space nor apoptotic area.

3.0 SAFETY INFORMATION

3.1 Continued absence of replication competent infectious virus was determined from sequential infection experiments. No replicative virus was detectable by PCR analysis of DNA samples from HeLa cells treated with the frozen/thawed cell extracts from HeLa cells initially infected with Ad5CMV-p53 at 100 PFU/cell, Ad5CMV-p53 was confirmed as a replication-defective and helper-independent virus. Further confirmation of this was obtained by labeling viral supernatants with [^{3}H]Thymidine. Absence of labeling in extracted DNA showed absence of replication competent adenovirus. These studies and the following safety studies will be performed by Microbiological Associates, Inc.

3.2 Sterility will be assured by testing for aerobic and anaerobic bacteria, fungus, and mycoplasma. Other tests to be performed by Microbiological Associates, Rockville, MD include:

Transmission EM for Viruses
In Vitro Assay for Adventitious Viral Contaminants
In Vivo Assay for Adventitious Viral Contaminants
Isoenzyme & Cytogenetic Analysis
Tumorigenicity$_c$
EBV
CMV
Hepatitis
HIV Co-Cultivation
HTLV 1/2 PCR
Adeno-Associated (AAV) Hybridization
Parvovirus B-19 Hybridization Adenovirus

4.0 PATIENT ELIGIBILITY

4.1 Patients must have histologic proof of non-small cell lung cancer. Patients must be either unable to receive conventional treatment (e.g. the patient received radiation therapy for an unrelated cancer in the same field and has inadequate pulmonary function for surgery) or have failed conventional treatment. Patients need not have received a trial of chemotherapy prior to entering this protocol.

4.2 Patients must have either an endobronchial tumor accessible by the bronchoscope, with some clinical evidence of bronchial obstruction, advanced local-regional cancer which is unresectable, or malignant pleural effusion.

4.3 All patients must have a life expectancy of at least 12 weeks and must have a performance status of $\leq$2 (Zubrod scale, Appendix B).

4.4 All patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable form is the one attached at the end of this protocol.

4.5 A tumor biopsy must show a p53 mutation by single-strand conformation analysis[28]. Material obtained previously and embedded in paraffin may be analyzed. If a new biopsy is required, the patient will be entered into the protocol and informed consent obtained if protocol entry is the sole reason for the biopsy. Mutations of the *p53* gene will be determined by SSCP analysis of exons 5-8 of PCR amplified tumor DNA.

4.6 Patients will be tested for HIV prior to entry onto the protocol and must be HIV-negative. Patients with upper respiratory infections will not be treated until the infection resolves.

4.7 Patients must have adequate bone marrow function (defined as peripheral absolute granulocyte count of >2,000/mm^3 and platelet count of 100,000/mm^3), adequate liver function (bilirubin $\leq$1.5 mg/dl), and adequate renal function (creatinine <1.5 mg/dl).

5.0 TREATMENT PLAN

5.1 The study will be an open-label upward dose ranging study for adenovirus-*p53* vector (Ad5CMV-p53).

5.2 The study will be done in two phases. It is not known what toxicities if any will be caused by the adenovirus. The first phase of the study will allow assessment of toxicities related only to the vector. Patients will receive one intratumor or intrapleural injection of Ad5CMV-p53. The initial dose will be 10^6 plaque forming units (PFU).

5.3 Following completion of the first vector-alone group, a second phase will evaluate Ad5CMV-p53 and cisplatin administered concurrently. Patients in the second group will receive one intratumor or intrapleural injection of Ad5CMV-p53 concurrent cisplatin at 30 mg/m^2 with 2 additional doses of cisplatin on days 2 and 3.

5.4 Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitation imposed by production of the adenovirus).

5.5 **Dose Escalation:** The adenovirus dose will increase in one $\log_{10}$ increments for each group. Patients entered at a given dose level will not be eligible for dose escalation. Dose escalation will proceed separately for the intratumor injection group and the intrapleural injection group. The dose of cisplatin will remain constant. We estimate based on the achievable titers of adenovirus that six dose levels can be done thus requiring 21 patients each for the intratumor and intrapleural groups for each of the two groups (Ad5CMV-p53 alone and Ad5CMV-p53 plus cisplatin).

5.6 All patients shall be registered with the Data Management office at 713-792-2926. At the time of registration, a prestudy form shall be necessary on each patient. When applicable, information pertaining to important prognostic factors such as tumor histology, pretreatment weight loss, performance status, disease stage and extent, and prior therapy will be requested.

5.7 Patients with local-regional tumors will have injection of a total dose of 10 ml for tumors $\geq$ 4 cm in diameter or 3 ml for tumors < 4 cm in diameter of the adenovirus preparation with the appropriate number of viral particles at multiple sites percutaneously under fluoroscopic guidance. Direct thoracoscopic injections may also be used.

5.8 Patients with pleural effusion will have a Hickman chest catheter inserted to drain the effusion and then will have 10 ml of the adenoviral preparation instilled into a chest catheter followed by 15 ml of normal saline. The chest catheter will be clamped for 3 hours with the patient turned from side-to-side. The chest catheter will then be unclamped and left to drainage.

5.9 For patients with obstructing tumors of the bronchus, patients will undergo bronchoscopy to assess the degree of obstruction. As much gross tumor as possible will be resected endoscopically. Patients may also have had brachytherapy as a tumor reduction modality. Patients will undergo bronchoscopy under topical or general anesthesia. A Stifcor™ transbronchial aspiration needle (21g) will be passed through the biopsy channel of the bronchoscope. The residual tumor site will be injected with 10^6 PFU of the adenovirus supernatant as the starting dose level. The volume will be no greater than 3 ml. Injections will be circumferential and will be intratumor and submucosal.

5.10 The treatment will be repeated monthly. Dose escalation may proceed after a minimum two week follow-up of the last patient entered into the previous dose level. Treatment will continue as long as there is no tumor progression. After one year the patients will be evaluated for continuation of therapy.

5.11 Patients will wear a surgical mask for 24 hours following injection of the adenovirus. All medical personnel will wear masks and face shields routinely during bronchoscopy and injection of the adenovirus. Anti-tussives will be prescribed as necessary. All patients will be kept in isolation during the time they are receiving injections of the adenovirus and for 48 hours after the last injection.

5.12 **Chemotherapy**
Dose Schedule: The dose levels and schedule will be as follows:

DRUG	DAYS	DAILY DOSE LEVELS (mg/m^2 IV)			
		-2	-1	0	
Cisplatin	1-3	20	25	30	

Level 0 represents the starting dose in all patients. Chemotherapy will be repeated every 30 days provided patient has recovered from all toxicities and lab work has normalized (absolute granulocyte count $\geq$1,800/mm^3, platelet count $\geq$100,000/mm^3, and creatinine $\leq$ 1.5 mg/dl). Cisplatin will not be given if grade IV neutropenia develops at level -2.

Cisplatin: Will be diluted in 500 cc NS + 8 meq $MgSO_4$ and administered IV over 2 hours daily on days 1-3. Hydration with 500 cc of D5W+1/2 NS + 8 meq MgSO4 given over 3 hours pre- and post-cisplatin infusion.

All patients should be receive appropriate antiemetics.

5.12.1 Cisplatin dosage changes for the subsequent course shall be based on the myelosuppression and other toxicity encountered in the preceding course. The dose modification will be as follows:

5.12.1.1

Lowest Absolute Granulocyte count		Lowest platelet count	Next Course Cisplatin
$\geq$500	and	$\geq$50,000	No change
<500	or	<50,000	-1 dose
neutropenic fever	or	bleeding	-1 dose

5.12.1.2

Non-hematologic toxicity

grade 3	-I level
grade 4	discontinue cisplatin

Creatinine must be $\leq$1.5 mg/dl before resuming chemotherapy.

6.0 PRE-TREATMENT EVALUATION

6.1 A complete history and physical to include performance status, recent weight loss, usual weight and concurrent non-malignant disease and its therapy, and all prior anticancer treatments must be recorded.

6.2 Laboratory studies shall include quantitative immunoglobulins; a CBC with differential and platelet count; SMA-12 and electrolytes, including creatinine, bilirubin, SGPT, alkaline phosphatase, urinalysis, and chest x-ray.

6.3 Any residual toxicity from prior therapies should be recorded using the grading schema in Appendix C.

6.4 Appropriate studies should be obtained to fully define the extent and severity of existing or suspected malignant and non-malignant disease.

6.5 Measurements of disease that can be measured on the chest roentgenogram will be documented. The location and size of the endobronchial lesion must be recorded prior to treatment. A photograph of the area will be taken at a fixed distance from the lesion. The area will be measured in 2 dimensions (product of longest and perpendicular dimensions).

6.6 A pretreatment blood sample will be collected and stored for analysis of leukocytes and serum.

7.0 EVALUATION DURING STUDY

7.1 Patients will have a CBC, platelet count, PT, PTT, SMA-12, electrolytes, and a chest x-ray prior to each course of therapy. Serum will be collected pre- and post-treatment for analysis of antibodies to adenovirus proteins.

7.2 History and physical with performance status and weight should be recorded before each course.

7.3 The endobronchial tumors will be photographed bronchoscopically at the beginning of each course. Tumor measurements are to be recorded before each course for all measurable tumors.

7.4 All relevant information regarding drug dosage, tumor response, laboratory examinations, and treatment-related toxicities must be recorded before each treatment is given.

7.5 Parameters to be Measured In Vitro

7.5.1 Fine needle aspirates or core biopsies will be obtained of accessible local tumor. Cells in the cytospins of pleural effusions will be obtained pre- and post-treatment when possible. Tumor specimens will be collected 72 hrs. after injection of the adenovirus during the first treatment cycle. For endobronchial tumors bronchoscopic tumor and normal bronchial epithelial biopsies will be obtained prior to the beginning of each course. Tissue will be fixed immediately in 4% paraformaldehyde and 0.5% gluteraldehyde at 4°C. This will permit extraction of DNA and RNA and permit in situ hybridization.

7.5.2 Biopsies will be analyzed for incorporation of the transduced gene into the host genomic DNA and expression of the transduced gene at the RNA level by standard hybridization techniques following polymerase chain reaction and by in situ hybridization.

7.5.3 All patients will be evaluable for response and toxicity following one course of therapy.

7.6 An autopsy will be requested on all patients enrolled in the protocol who die. DNA will be extracted from tumor and normal tissues to determine if the adenovirus genes are present and expressed. PCR amplification of specific sequences will be used to determine this.

7.7 A blood sample will be collected three times at one-half hour intervals following injection of the adenovirus. These samples will provide leukocytes to analyze for uptake of adenovirus DNA. Serum will be tested for antibodies to adenovirus proteins. This will be done by western blot analysis performed by Microbiological Associates, Inc. (Rockville, MD). Patients will be tested monthly during treatment, monthly for the first three months following completion of treatment, every three months for the remainder of the year following completion of treatment, and then at least yearly thereafter.

7.8 Normal tissue samples will be collected during the follow-up visits and bronchoscopies. These will include samples of normal bronchial mucosa, leukocytes, and germ cells, if possible. These tissues will be analyzed for incorporation of the adenovirus.

8.0 CRITERIA FOR RESPONSE AND TOXICITY

8.1 The graded toxicity scale used in this study has been previously described[29]. Three patients will be initially entered at each dose level. If one patient in the cohort of 3 develops grade 3 toxicity for any system except hematologic (grade 4 required), an additional 3 patients will be entered at that dose level. If 2 of the 6 patients develop grade 3 (grade 4 hematologic) or greater toxicities, the next lower dose level not causing these toxicities is declared the Maximum Tolerated Dose (MTD). If MTD toxicity were to occur, patients could continue treatment at the next lower dose level. The MTD will be determined separately for each phase of the study. If the MTD is reached with the adenovirus plus cisplatin phase but not with the adenovirus alone phase, the study may continue with the adenovirus alone. If MTD toxicity occurs in a cohort of 3 patients, then the next 3 patients may

bypass the adenovirus alone phase and initially receive the adenovirus and cisplatin to establish the MTD for this combination.

8.2 The tumor bed will be photographed prior to each course of therapy for endobronchial tumors.

8.3 The longest diameter and its perpendicular will be measured will be determined for measurable lesions. Size will be reported as the product of the diameters. For pleural effusions reduction in the volume of the effusion and absence of malignant cells will be noted. Patients with pleural effusions or endobronchial tumors will not be considered evaluable for an objective response.

8.4 The rate of regrowth of the tumor will be calculated from the above measurements.

8.5 Patients will be evaluable for response if they had received at least one course of chemotherapy followed by an appropriate radiograph to document response. A complete response was defined as disappearance of all clinical evidence of tumor without the appearance of new lesions for a period of at least four weeks. Patients evaluable for a less-than-complete response must have had a bidimensionally measurable tumor. Partial response was defined as a 50% or greater reduction in the sum of the products of the diameters of the measurable disease; a minor response was defined as a 25% to less than 50% reduction in the sum of the products of the diameters of the measurable lesion. Patients were designated as having progressive disease if they showed a 25% or greater increase in the size of their disease or if they developed unequivocal new lesions during treatment, and having no change if they had any tumor change not meeting the criteria described above.

8.6 The time to progression will be measured from the first observation with reduction in tumor bulk until there is evidence of progressive disease. Progressive Disease is defined as an increase of $\geq$ 25% in the sum of the products of the diameters of the measured lesion. Patients must have received at least two courses of therapy before a designation of progression is made. The survival of patients will be measured from entry into protocol.

8.7 All toxicities encountered during the study will be evaluated according to the grading system (0-4) in Appendix C and recorded prior to each course of therapy. Duration of the toxicity and its treatment will be recorded. Life-threatening toxicities should be reported immediately to the Study Chairperson, who in turn, must notify the IRB, RAC, and FDA.

9.0 CRITERIA FOR DISCONTINUING THERAPY

9.1 Presence of obstructing endobronchial tumor that has recurred after a minimum of 2 courses of treatment.

9.2 The development of unacceptable toxicity defined as unpredictable, irreversible, or Grade 4 (non-hematologic). Patient refusal of therapy due to a specific toxicity should be graded as 4 and an explanatory note recorded.

9.3 Non-compliance by patient with protocol requirements.

9.4 Patient refusal to continue treatment.

9.5 Criteria for removal from protocol:
- a) Refusal to continue study participation
- b) Significant hemoptysis
- c) Coagulopathy
- d) Progressive postobstructive pneumonia

10.0 DATA AND PROTOCOL MANAGEMENT

10.1 Protocol Compliance: The attending physician and oncology research nurse must see patients prior

to drug administration. All required interim and pretreatment data should be available and the physician must have a designation as to tumor response and toxicity grade.

10.2 Data Entry: Data must be entered into the Clinical Data Management System before a course of therapy can be given. A brief explanation for missing data should be recorded as a comment.

10.3 Accuracy of Data Collection: The Study Chairperson will be the final arbiter of response of toxicity should a difference of opinion exist.

11.0 STATISTICAL EVALUATION

Three patients will be entered at each dose level with 6 patients entered at the maximum tolerated or maximum attainable dose (limitations imposed by production of the adenovirus). A maximum of 21 patients will be entered in the intratumor and the intrapleural groups, respectively for each of the treatment groups (Ad5CMV-p53 alone and Ad5CMV-p53 plus cisplatin).

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THE UNIVERSITY OF TEXAS M. D. ANDERSON CANCER CENTER

INFORMED CONSENT

Protocol Title: **Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis (cancer cell death) in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wildtype (normal) p53 and Cisplatin**

1. _____ _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so that you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other consents you may have signed.

DESCRIPTION OF RESEARCH

2. PURPOSE OF THE STUDY: Some lung cancers seem to arise because problems occur in a part of the cells (a particular gene called "p53") that usually stops cancer. In this clinical research study the investigators are trying to insert a normal copy of the p53 gene into the lung cancer cells to see if this will cause the cancer cells to stop growing. As a "vector" that is, as a vehicle to get the p53 gene into the cells, the investigators will use an adenovirus (related to cold viruses) that has been altered so it cannot reproduce itself. Because the investigators are at an early stage in the research, the investigators do not expect to cure the patients who participate; instead, the immediate purpose of the study is to find out what dose of vector can be used without harming patients and whether the new p53 genes will cause cancer cells to stop growing.

3. DESCRIPTION OF RESEARCH: The experimental protocol is as follows: Patients for this study will be selected from among patients with non-small cell lung cancer who are not now candidates for radiation therapy or surgery. Patients with local tumors that cannot be removed with surgery or have recurred after radiation can participate. It is not necessary that the patient received chemotherapy (drugs) alone but chemotherapy may be an alternative treatment. These tumors will be directly injected with the adenovirus. Patients with fluid in the chest caused by tumor cells will also participate and will have the adenovirus directly injected in the fluid. Patients who have known tumors within their airway can also participate.

These patients are scheduled to have bronchoscopic exams as part of their evaluation either under local anesthesia or general anaesthesia. The surgeon performs an examination using a regular white light source to illuminate the tracheobronchial tree. If a tumor is found that is obstructing a portion of the tracheobronchial tree, that patient will be considered eligible for the study. It must also be possible to reduce the number of tumor cells to a small amount with removal through the bronchoscope or laser treatment.

The tumor must have a specific gene mutation in it. It may be possible to find this mutation in specimens from previous biopsies. However, if these are not available new biopsies must be performed. If the new biopsy material does not show the mutation, the patient cannot participate in the study. The residual tumor will be injected once with an adenovirus containing a gene that possibly can correct an abnormality detected in the cancer cells. This treatment will be repeated monthly as long as there is evidence that the tumor is not growing. The cost of the routine bronchoscopy and biopsies which are part of the work up of patient's condition shall be his/her responsibility. The patient will receive cisplatin, a drug, along with the adenovirus. The patient will be treated for 3 days with a drug called cisplatin which is a chemotherapeutic agent. This will assist the adenovirus in killing the tumor cells. A maximum of 21 patients will be entered in the intratumor and the intrapleural groups, respectively.

The investigators wish to follow the patient's medical condition for the rest of the patient's life. If the patient should move, the patient should notify Dr. Roth's office.

PERMISSION FOR AUTOPSY: In case of death, the family of the patient will be asked for an autopsy because an accurate documentation of the precise cause of death may be important to all future patients.

4. **RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:**

Two additional biopsies will be required in addition to the initial biopsy. A bronchoscopic biopsy samples a very tiny piece of the mucosa. Risks of biopsies include hemoptysis (coughing up blood) which is usually transient and self limited. Severe hemorrhage requiring emergency maneuvers including intubation and thoracotomy are exceedingly rare. Although extremely rare, any experimental procedure may result in unforeseen risks. Appropriate medical therapy will be made available for the treatment of such side effects.

4a. This clinical procedure may involve unforeseeable risks to unborn children; therefore, the participants should practice adequate methods of birth control throughout the period of their involvement in the clinical study if they are sexually active. To help prevent injury to children, the female participants should refrain from breast feeding during participation in the clinical research study.

4b. *Possibility of Causing a New Cancer.* It is possible that the research could cause cancer in normal cells but the investigators believe the risk is very small particularly because the investigators test the material that is injected to make sure it does not contain disease-causing viruses.

4c. *Side Effects of Chemotherapy.* The usual expected side effects of this treatment include nausea and vomiting, hair loss, mouth ulcers, skin rash, or diarrhea. Toxic effects to the bone marrow cells may occur, and may make a patient more susceptible to serious infections or bleeding. Additional side effects may include: kidney damage, hearing loss, nerve damage which may cause numbness or tingling. Since there is no experience with the adenovirus and chemotherapy combination and since no other patients have received this virus, there is a possibility of unexpected, severe, or even permanent damage to any body organ. All patients will be closely monitored for the development of complications and every attempt will be made to prevent them.

5. POTENTIAL BENEFITS: The primary purposes of this study are scientific namely, to test the safety of the procedure and to see whether the injected gene will function in the cancer cells. If this happens, there might be a potential benefit to subjects, depending on how much of their cancer is destroyed. The main benefit expected is advancing knowledge and developing future studies and treatments rather than curing the patient-subjects who participate in this study, although that is always a possibility.

6. ALTERNATE PROCEDURES OR TREATMENTS: Patients will be eligible for this study if conventional therapy such as surgery or external beam radiation therapy has already been performed or cannot be done. Alternatively chemotherapy alone can be given.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the procedure involved and the investigator has been willing to reply to my inquiries. This procedure will be administered under the above numbered, titled and described clinical research protocol at this institution. I hereby authorize Dr. Jack A. Roth, the attending physician/investigator and

designated associates to administer the treatment.

8. I have been told and understand that my participation in this clinical research study is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

   Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or effects of my decision with my physician.

   In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

   I will be informed of any new findings developed during the course of this clinical research study which might be relevant to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration, Microbiological Associates, Magenta Corporation (manufacturers of the virus), and National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.

10. I have been informed that, should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available for treatment at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for such injury.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of the study, I understand that I may be required to cover the cost of subsequent doses.

Costs related to my medical care including expensive tests or procedures that may be required by this clinical research study shall be my responsibility unless the sponsor or other agencies contribute toward said costs. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, devices, new drugs, or possible patentable procedures, in which event I understand that I cannot expect to receive any compensation or benefits from the subsequent use of information acquired and developed through may participation in this research project.

13. I understand that refraining from breast feeding and practicing effective contraception is medically necessary and a prerequisite for my participation in this clinical research study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.

14. I may discuss any questions or problems during or after this study with **Dr. Jack A. Roth at (713) 792-6932.** In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman or the Surveillance Committee at (713) 792-3220 and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based upon the above, I consent to participate in the research and have received a copy of the consent form.

_____ _____

DATE **SIGNATURE OF PARTICIPANT**

_____ _____

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR **SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP**

I have discussed this clinical research study with the Participant and/or his or her authorized representative using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe the participant understood this explanation.

_____

PHYSICIAN/INVESTIGATOR

Jack A. Roth, M.D. Professor and Department Chairman

Cornell Univ, Ithaca, NY	B.A.	1967	Economics
Johns Hopkins Univ Sch of Med, Baltimore	M.D.	1971	Medicine
UCLA Sch of Medicine, Los Angeles, CA	Fellowship	1971-1975	Res Surg Oncol
UCLA Sch of Medicine, LA	Residency	1972-1980	Surg/Thorac Surg

1980—86	Senior Investigator, Surgery Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland
1980—86	Lecturer in Surgery, Johns Hopkins Univ Sch of Medicine, Baltimore, Maryland
1982—86	Clinical Assistant Professor, Department of Surgery, School of Medicine, The Medical Center, Georgetown University, Washington, D.C.
1982—86	Head, Thoracic Oncology Section, Surgery Branch,Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
1986—P	Bud S. Johnson Professor of Thoracic Surgery and Chairman, Department of Thoracic Surgery, UT M. D. Anderson Cancer Center, Houston, Texas
1986—P	Professor of Tumor Biology, Department of Tumor Biology, M. D. Anderson Cancer Center, Houston, TX
1987—P	Faculty Appointment, Graduate School of Biomedical Sciences, The University of Texas Medical Sch, Health Science Ctr, Houston, TX
Nov 1991-Aug 1992	Director of the Lung Cancer Research Program, Dept of Thoracic Surgery, in the Division of Surgery, UTMDACC, Houston, Texas

Honors and Awards:

1967	B.A. Magna cum Laude with distinction in all subjects, Phi Beta Kappa, Cornell University, Ithaca, New York
1971	Outstanding Student Research Presentation, Johns Hopkins Medical Society
1975	James Ewing Society Resident—Fellow Clinical Research Award
1977	Society of Surgical Oncology Resident—Fellow Award
1981	Special Achievement Award, U.S. Department of Health and Human Services
1983	Who's Who in Frontier Science and Technology
1987	Recipient of Merit Award, Grant R01 CA45187 from Division of Research Grants and Biologic Response Modifiers Program, Nat'l Institutes of Health
1990	Who's Who in Health and Medical Services
1991	Who's Who in Science and Engineering
1991	The Best Doctors in America
1992	Lucy Wortham James Basic Research Award (SSO)

Selected Publications:

Yang SC, Owen—Schaub L, Mendiguren—Rodriguez A, Grimm EA, Hong WK, **Roth JA**: Combination immunotherapy for non—small cell lung cancer: Results using interleukin—2 and tumor necrosis factor—α. J Thorac Cardiovasc Surg 99:8-13, 1990.

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Slater JD, Ellerbroek NA, Barkley HT, Mountain C, Oswald MJ, **Roth JA**, Peters LJ: Radiation therapy following resection of non—small cell bronchogenic carcinoma. Intern J Radia Oncol, Biol, Phys. 20:945-951, 1991.

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Putnam EA, Yen N, Gallick GE, Steck PA, Fang K, Akpakip B, Gazdar AF, **Roth JA**: Autocrine growth stimulation by transforming growth factor-α in human NSCLC. J Surg Oncol 1:49-60, 1992.

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Casson AG, Putnam JB, Natarajan G, Johnston DA, Mountain C, McMurtrey M, **Roth HA**: Five year survival after pulmonary metastases for adult soft tissue sarcoma. Cancer 69(1):662- 668, 1992.

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Zhang Y, Mukhopadhyay T, Donehower L, Georges R, **Roth JA**: Retroviral vector-mediated transduction of k-ras antisense RNA into human lung cancer cells inhibits expression of the malignant phenotype. Human Gene Therapy 4:451-460, 1993.

HUMAN GENE TRANSFER PROTOCOL

9406-080 Sobol, Robert and Royston, Ivor; San Diego Regional Cancer Center; San Diego, California; *Injection of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL-2): A Phase I Study.*

Date of RAC Approval: 6-10-94
Date of NIH Approval: 7-12-94

SCIENTIFIC ABSTRACT

The overall objective of this project is to perform a Phase I Clinical Trial in glioblastoma patients of cytokine gene transfer comprising subcutaneous injections of autologous or allogeneic HLA-A2 matched tumor cells genetically modified to secrete interleukin-2 (IL-2). The Phase I study will evaluate the safety, anti-tumor effects and immune responses induced by subcutaneous injections with escalated doses of irradiated HLA-A2 positive autologous or allogeneic tumor cells transduced with a replication incompetent IL-2 retroviral vector. We have treated one patient with glioblastoma multiforme who had failed conventional therapy with IL-2 gene therapy employing autologous tumor cells and fibroblasts transduced with an IL-2 retroviral vector. No significant morbidity was noted at the injection sites and post treatment monitoring of complete blood counts, serum chemistries and urinalyses revealed no significant changes from pre-treatment values. Cellular anti-tumor immune responses and tumor necrosis were observed during the course of therapy. This patient's tumor has an HLA-A2 positive haplotype and the IL-2 transduced tumor has been established as a continuous cell line. This cell line, termed GT9, will be utilized as the allogeneic HLA-A2 positive IL-2 transduced cell line for immunizations in this study. In the Phase I study, a group of patients will be injected with IL-2 transduced HLA-A2 positive autologous tumor cells and a second group will be treated with IL-2 transduced GT9 allogeneic irradiated tumor cells. Patient cohorts will receive injections with increasing doses of transduced tumor cells. All patients enrolled in the study will also have an established autologous tumor cell line for application in humoral and cellular immune response monitoring assays. The patients will be monitored for toxicity, anti-tumor responses and the induction of anti-tumor immunity. The results of the Phase I trial should permit an assessment of the safety of this form of cytokine gene therapy and provide initial data to evaluate the potential utility of IL-2 gene therapy with either autologous or partially HLA matched allogeneic tumor cells.

NON-TECHNICAL ABSTRACT

The overall objective of this project is to perform a clinical trial in glioblastoma patients comprising injections of tumor cells genetically modified to secrete an immunostimulatory substance termed interleukin-2 (IL-2). The study will evaluate the safety, anti-tumor effects and immune responses induced by injections with escalated doses of the irradiated, genetically modified tumor cells. We have treated one patient with glioblastoma multiforme who had failed conventional therapy with IL-2 modified cells. No significant toxicity was noted at the injection sites and post treatment monitoring of blood and urine tests of organ function revealed no significant changes from pre-treatment values. Anti-tumor immune responses were observed during the course of therapy. This patient's tumor, termed GT9, will be utilized to inject other patients. In this study, a group of patients will be injected with their own irradiated tumor cells which have been genetically modified to secrete IL-2 and a second group of patients will be treated with IL-2 gene modified irradiated GT9 cells. Patient groups will receive injections with increasing doses of the genetically modified tumor cells. The patients will be monitored for toxicity, anti-tumor responses and the induction of anti-tumor immunity. The results of the trial should permit an assessment of the safety of this form of IL-2 gene transfer and provide initial data to evaluate the potential utility of IL-2 gene therapy in glioblastoma patients.

CLINICAL PROTOCOL

Injection of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL-2): A Phase 1 Study

--4/94

STUDY CO-CHAIRS:
Robert E. Sobol, M.D.
Ivor Royston, M.D.
San Diego Regional Cancer Center
3099 Science Park Road, Suite 200
San Diego, CA 92121
Telephone: (619) 450-5990/Fax: (619) 450-3251

CO-INVESTIGATORS:
Habib Fakhrai, Ph.D.
Daniel L. Shawler, B.S.
Ruth Gjerset, Ph.D.

1.0 Objectives

1.1 Evaluate the safety of subcutaneous injections with irradiated autologous or allogeneic HLA-A2 positive glioblastoma cells genetically modified to express the gene for IL-2.

1.2 Examine the effects of these injections on tumor growth *in vivo*.

1.3 Assess whether the injections induce cellular or humoral anti-tumor immune responses.

1.4 Compare the anti-tumor effects and immune responses induced by injections with genetically modified HLA-A2 positive autologous versus allogeneic tumor cells.

2.0 Background/Rationale

2.1 Interleukin-2 Cancer Immunotherapy in Human Subjects

Recent advances in our understanding of the biology of the immune system have lead to the identification of important modulators of immune responses (1-3). These agents mediate many of the immune responses involved in anti-tumor immunity. Several of these cytokines have been produced by recombinant DNA methodology and evaluated for their anti-tumor effects. In experimental clinical trials, the administration of cytokines and related immunomodulators has resulted in objective tumor responses in patients with various types of neoplasms (4-7).

Interleukin-2 (IL-2) is an important cytokine in the generation of anti-tumor immunity (4). In response to tumor antigens, a subset of lymphocytes termed helper T-cells secrete small quantities of IL-2. This IL-2 acts locally at the site of tumor antigen stimulation to activate cytotoxic T-cells and natural killer cells which mediate systemic tumor cell destruction. Intravenous, intralymphatic or intralesional administration of IL-2 has resulted in clinically significant responses in some cancer patients (4-6). However, severe toxicities (hypotension and edema) limit the dose and efficacy of intravenous and intralymphatic IL-2 administration (5,7). The toxicity of systemically administered cytokines is not surprising since these agents mediate local cellular interactions and they are normally secreted in only very small quantities.

To circumvent the toxicity of systemic IL-2 administration, several investigators have examined intralesional injection of IL-2. This approach eliminates the toxicity associated with systemic IL-2 administration (8,9). However, multiple intralesional injections are required to optimize therapeutic efficacy (8,9). These injections will be impractical for many patients, particularly when tumor sites are not accessible for direct injection without potential significant morbidity.

The aim of this study is to demonstrate the safety of a novel and more practical method of IL-2 cancer immunotherapy. In this approach, a patient's tumor cells are genetically modified to express and secrete IL-2. The genetically modified cells are then employed in subcutaneous immunizations to induce systemic anti-tumor immunity.

This approach provides the benefit of local IL-2 administration and obviates the need for multiple intralesional injections. The amount of IL-2 secreted by the genetically modified cells is sufficient to induce anti-tumor immunity but is too low to produce systemic toxicity. In addition, the continuous expression of functional amounts of IL-2 may also produce greater augmentation of anti-tumor immune responses compared to that obtained by intermittent cytokine injections.

2.2 Cytokine Gene Therapy in Animal Tumor Models

Cytokine gene transfer has resulted in significant anti-tumor immune responses in several animal tumor models (10-13). In these studies, the transfer of cytokine genes into tumor cells has reduced or abrogated the tumorigenicity of the cells after implantation into syngeneic hosts. The transfer of genes for IL-2 (10,11), gamma interferon (IFN) (12), or IL-4 (13) significantly reduced or eliminated the growth of several different histological types of murine tumors. No toxicity associated with expression of the cytokine transgenes was reported in these animal tumor studies (10-13). In the studies employing IL-2 gene transfer, the treated animals also developed systemic anti-tumor immunity and were protected against subsequent tumor challenges with unmodified parental tumor (10,11). Similar inhibition of tumor growth and protective immunity were also demonstrated when immunizations were performed with a mixture of unmodified parental tumor cells and genetically modified tumor cells engineered to express the IL-2 gene. We have successfully induced anti-tumor immunity in a model of colorectal carcinoma by immunization with IL-2 transduced cells. Immunization with a mixture of irradiated tumor cells and IL-2 transduced cells induced systemic anti-tumor immunity capable of rejecting a subsequent live tumor cell challenge. Repeated immunizations with a mixture of irradiated tumor cells and IL-2 transduced fibroblasts abolished established, visible tumors in a subset of the treated animals (Appendix 12.7). Glioblastomas are known to secrete immunosupressive factors which compromise host anti-tumor immune responses and glioblastoma patients have depressed CD4+ helper T cells (19). Several studies, including our own have indicated the ability of IL-2 transduced cells to bypass the need for CD4+ helper T cells in generating efficacious cellular immunity (10,11, Appendix 12.7).

2.3 Immunotherapy of Gliomas

The potential utility of active immunotherapy for the treatment of gliomas was suggested by a study performed Mahaley et. al (16). In this investigation, patients received monthly subcutaneous immunizations with human glioma tissue culture cell lines. Patients were also treated with levamisole and received radiotherapy and BCNU chemotherapy. Patients inoculated with the U-251MG cell line had significantly longer survival compared to non-immunized historical control patients treated with levamisole, radiotherapy and chemotherapy. There was no clinical evidence of allergic encephalomyelitis in the immunized patients and no pathological findings of this disorder on gross or light microscopic evaluations performed at autopsy in a subset of patients.

Since the performance of these studies nearly a decade ago, there have been significant advances in our knowledge of the immune system and in our ability to modulate immune responses. This has led to the development of novel experimental immunotherapies for cancer. In more recent animal studies of gliomas, immune rat cytotoxic T cells primed *ex vivo* against a rat glioma were given intravenously to rats with intracerebral glioma and induced regression of these brain tumors (17). In the C6 rat glioma model, effective treatment of intracranial tumors was observed following immunizations with tumor cells genetically modified to inhibit insulin like growth factor (IGF-1) gene expression by plasmids encoding IGF-1 anti-sense nucleotides (18). The results of these studies document the ability of systemically generated immune effector cells to inhibit the growth of intracranial glioma tumors (17,18). Furthermore, adoptive immunotherapy studies with primed lymphocytes in rodent glial tumor models have demonstrated the requirement for IL-2 to produce efficacious anti-tumor responses (17,20). These findings further support the proposed use of IL-2 transduced tumor cells.

2.4 Gene Transfer into Human Subjects

The methods we will utilize to transfer cytokine genes into cells are virtually identical to those employed in previously approved human gene transfer studies in which tumor infiltrating lymphocytes (TILs) were modified by retroviral gene mediated transduction and administered to cancer patients (14). In this earlier Phase I study of retroviral mediated gene transfer, TILs were genetically modified to express the neomycin phosphotransferase (neo^R) gene. Following intravenous infusion, polymerase chain reaction analysis consistently demonstrated the presence of genetically modified cells in the circulation for as long as two months after administration. No infectious retroviruses were identified in these patients and no side effects due to gene transfer were noted in any patients (14). Additional Phase I clinical protocols employing similar retroviral gene transfer methods have recently been approved by the Recombinant DNA Advisory Committee (RAC). Our Phase I study of human cancer patients will utilize the same gene transfer methods and safety testing procedures employed in these previously approved investigations.

2.5 Single Patient IL-2 Gene Therapy of Glioblastoma

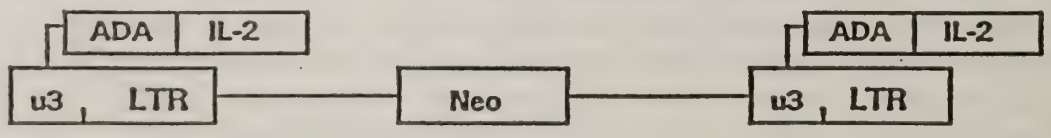
2.5.1. Patient History

We have treated one glioblastoma multiforme (GBM) patient with IL-2 gene therapy. The patient is a 52 year old female with GBM of the right temporal lobe diagnosed in 1/92. She was initially treated with surgical resection, conventional radiotherapy and PCV chemotherapy (procarbazine, CCNU and vincristine). Nine months later, a second resection was performed for tumor recurrence. Tumor pathology revealed a GBM at re-resection. The patient's tumor progressed after experimental treatment with accutane and with an I^{131} radioisotope labeled anti-tenacin monoclonal antibody. Subsequently, the patient was treated with experimental stereotactic radiation therapy designed to encompass the site of tumor involvement.

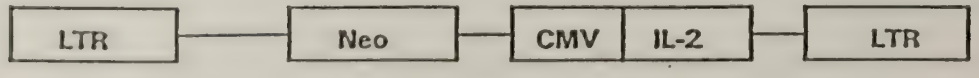
2.5.2. IL-2 Gene Therapy

IL-2 gene therapy was initiated in January 1993, approximately one year after the first tumor resection. The patient received nine subcutaneous injections at 2 to 4 week intervals with either autologous, irradiated IL-2 transduced tumor cells or a mixture of irradiated tumor cells and irradiated IL-2 transduced fibroblasts. A tenth iinjection was performed approximately 3 months after the ninth inoculation. Two IL-2 retroviral vectors were employed in this study. The retroviral vector DC/AD/R/IL-2 (kindly provided by Dr. Bernd Gansbacher) utilized an adenosine deaminase promoter to drive IL-2 expression while the retroviral vector G1NaCvi2.23 (kindly provided by Genetic Therapy Inc.) employed a cytomegalovirus promoter (Figure 1).

Figure 1



Retroviral Vector DC/ADA/R/IL-2



Retroviral Vector G1NaCvi2.23

Table 1 lists the transduced cell types and IL-2 doses administered for eachinjection. IL-2 secretion by the transduced cells in vitro was measured by an enzyme linked immunosorbent assay (ELISA). Tumor cells transduced with DC/AD/R/IL-2 and G1NaCvi2.23 were found to express similar amounts of IL-2 in vitro (10-43 units IL-2/10^6 cells/24 hrs). However, fibroblasts transduced with the G1NaCvi2.23 vector secreted approximately 5 to 10 fold higher levels of IL-2 compared to those transduced with the DC/AD/R/IL-2 vector (Table 1). The total administered IL-2 dose ranged from 3 to 440 units/24 hrs. The total tumor cell dose for each injection was 10^7 cells.

Table 1 **IL-2 GENE THERAPY OF GLIOBLASTOMA TREATMENT SUMMARY**

Date of Immunization Rx #	Retroviral Vector	Transduced Cell Type	# of Transduced Cells	IL-2 Secretion 10^6 cells/24 hr	Total Dose of IL-2
1/4/93 Rx # 1	DC/AD/R/IL-2	tumor	1.25×10^5	43 units	5 units
1/27/93 Rx # 2	DC/AD/R/IL-2	tumor	2.60×10^5	10 units	3 units
2/19/93 Rx # 3	DC/AD/R/IL-2	fibroblast	4.60×10^5	40 units	18 units
3/5/93 Rx #4	DC/AD/R/IL-2	fibroblast	4.20×10^5	51 units	21 units
3/26/93 Rx #5	DC/AD/R/IL-2	fibroblast	2.25×10^5	35 units	8 units
4/16/93 Rx #6	DC/AD/R/IL-2	fibroblast	1.20×10^5	91 units	11 units
5/12/93 Rx #7	G1NaCvi2.23	fibroblast	2.50×10^5	427 units	107 units
5/27/93 Rx#8	G1NaCvi2.23	fibroblast	3.20×10^5	328 units	125 units
		tumor	2.00×10^6	10 units	
6/11/93 Rx#9	G1NaCvi2.23	fibroblast	2.00×10^5	550 units	440 units
		tumor	1.00×10^7	33 units	
9/17/93 Rx#10	G1NaCvi2.23	fibroblast	4.40×10^5	135units	112 units
		tumor	2.90×10^6	18 units	

2.5.3. Immune Responses

The patient's peripheral blood mononuclear cells and serum were analyzed to assess the development of cellular and humoral anti-tumor immune responses against autologous cultured tumor cells. As shown in Figure 2, peripheral blood mononuclear cells obtained between the 3thd and 8th injections frequently demonstated 3 to 4 fold greater tumor lytic activity compared to the pre-treatment control (there were insufficient viable cells to assess the effects of treatments 9 an 10). Tumor lytic activity following the 8th treatment could be completely inhibited by incubation of the effector cells with autologous tumor and partially inhibited by K562 cells (Figure 3). Partial inhibition of tumor lytic activity was observed with anti-CD8 but not with anti-CD4 antibody. These findings are consistent with the generation of a cellular anti-tumor immune response. The data suggest that this cellular immune response is comprised of both natural killer and CD8+ cytotoxic T cell components.

Since the last RAC submission, we have performed additional assays with cryopreserved samples from our previously treated patient to confirm the reliability of the assay results and to provide data on cellular immune responses at additional time points during the course of observation. Intra-assay coefficients of variation ranged from 0.8 to 6% indicating the reliability of the cellular immune response assays. Overall, repeat evaluations were performed with 10 specimens. Discordant values were observed for a single specimen--the inhibition of tumor lytic activity by K562 cells tested for the 8th treatment (Figure 3). The results 22.8 +/- 0.6 % and 3 +/- 3 % were obtained in different assays. We have chosen to plot the 22.8 +/- 0.6% value in Figure 3 as this value is most consistent with the concomittent demonstration of partial inhibition of tumor lytic activity by anti-CD8 antibody. The intra-class correlation coefficient was determined as a measure of inter-assay variation. The intra-class correlation coefficient was .65 for all data points and .90 if the discordant specimen was excluded from the analysis.

Additional assays were performed with peripheral blood obtained following 2 and 3 month no treatment intervals between treatments 9 and 10. These samples also exhibited low baseline levels of anti-tumor lytic activity similar to the levels obtained prior to the initiation of therapy and following injections with transduced cells secreting very low doses of IL-2 (less than 10 units IL-2/24 hours). In summary, negative results were obtained at 5 different time points spanning the first six weeks of observation (pre-treatment and post-treatments 1 and 2 with very low doses of IL-2) and following 2 and 3 month no treatment intervals between treatments 9 and 10. Hence, there was no evidence of anti-tumor cellular immune responses in the absence of treatment with genetically modified cells secreting > 20 units IL-2/24 hours. Furthermore, partial inhibition of cytolytic activity by anti-CD8 antibody following the 8th treatment with higher doses of IL-2 secretion suggests the generation cytotoxic CD8+ T cells as a component of the cellular anti-tumor immune response. These data support our contention that injection of the genetically modified cells was associated with the generation of a cellular anti-tumor immune response and that the observed cellular immunity was not due to variability in the assay procedures nor solely the result of natural fluctuations in the patient's anti-tumor cellular immune responses.

2.5.4. Clinical Course

There have been no significant adverse reactions at the immunization sites and no treatment related abnormalities have been observed on monitoring of complete blood counts, serum chemistries and urinalyses. Transient, mild erythema (<24 hrs) was observed at the injection site with immuninzations at

IL-2 doses >100 units/24 hrs. Therapy with tamoxifen (80 mg BID) was initiated approximately 3 months after the first immunization. This agent has inhibited tumor growth in some GBM patients. MRI scans performed at approximately 4 week intervals during the first five months of treatment revealed modest changes in overall tumor size with waxing and waning of peritumoral edema associated with alterations in decadron doses. The MRI scan performed six months after the initiation of treatment (4 weeks after the highest dose IL-2 immunization) revealed marked tumor necrosis with significant peritumoral edema (Figure 4). Clinically, these MRI findings were associated with an exacerbation of the patient's baseline left sided weakness. This gradually resolved following the administration of increased decadron doses which have been gradually tapered. Stereotactic intraventriculostomy was performed to relieve increased pressure in the left third ventricle. Cytological evaluation of cerebrospinal fluid revealed the presence of inflammatory cells without detectable tumor cells. The patient received no further treatment until 2 months later when the MRI scan revealed renewed tumor growth (8 months after the initiation of treatment). At this time, the patient was injected with a combination of transduced tumor cells and fibroblasts and non-transduced tumor cells to yield an IL-2 dose of 110 units/24 hrs. The clinical course of the patient is described in detail in Appendix 12.8.

In summary, IL-2 gene therapy resulted in no significant toxicity at the sites of immunization and was associated with the generation of a cellular anti-tumor immune response. Marked tumor necrosis was observed following the highest IL-2 immunization dose. It is not possible to draw meaningful conclusions regarding safety, efficacy or immune response induction from the results of a single patient. However, we are encouraged by these preliminary findings and believe that they warrant evaluation of IL-2 gene therapy in additional GBM patients as outlined in this protocol.

3.0 Treatment And Monitoring Schema

The treatment and monitoring schedules are summarized in Figures 5 and 6. After each injection, patients will be contacted daily for 3 days to determine if the injection site is swollen or red. If a history of any erythema or induration is elicited, then the patient will be examined within 24 hours. All subjects will be examined 14 days after each immunization. Physical examinations and laboratory evaluations will be performed every two weeks during the first 8 weeks of the study, once a month for three months and every 3 months for one year after the completion of therapy and at least yearly thereafter.

Figure 5 Treatment and Monitoring Summary

Day - 60	Tumor biopsies Genetic modification of tumor cells
Days + 0,14,28	Immunization with irradiated transduced tumor cells

Figure 6

TREATMENT AND MONITORING SCHEDULES

Treatment or Evaluation	Day of Study[1]										
	0	14	28	42	60	90	120	210	300	390	Yearly
Immunization	x	x	x								
History/Physical	x	x	x	x	x	x	x	x	x	x	x
Laboratory											
CBC	x	x	x	x	x	x	x	x	x	x	x
PT, PTT	x	x	x	x	x	x	x	x	x	x	x
Chem 20	x	x	x	x	x	x	x	x	x	x	x
CH50 C3, C4	x	x	x	x	x	x	x	x	x	x	x
UA	x	x	x	x	x	x	x	x	x	x	x
Viral/PCR Assays											
10cc Green Top	x	x	x	x	x	x	x	x	x	x	x
Serum IL-2 Humoral Immunity											
10 cc Red Top	x	x	x	x	x	x	x	x	x	x	x
Immunophenotyping Cellular Immunity Skin Testing											
50 cc Green Top	x	x	x	x	x	x	x	x	x	x	x

[1] The two week evaluations may be delayed up to 72 hours and the 3 month evaluations may be delayed by one week if necessary due to logistical/patient convenience issues.

4.0 Patient Selection and Registration

4.1 Eligibility

4.11 Age 18 years or older with no significatnt medical problems. Nonpregnant females.

4.12 Recurrent residual glioblastoma multiforme of the brain with measurable disease on enhanced CT or MRI. Imaging studies must be performed within two weeks of starting treatment.

4.13 HLA-A2 haplotype

4.14 Autologous tumor established as continuous culture in vitro.

4.15 Patients must have failed surgery and radiation therapy. Prior chemotherapy is not required. However, patients receiving prior treatment, including chemotherapy must wait 6 weeks before starting treatment. Patients with rapidly progressive tumors (e.g. at the rate that indicates that waiting 6 weeks is medically inadvisable) may enter treatment if adverse effects of prior treatment have been resolved.

4.16 Expected survival must be at least 3 months.

4.17 Karnofsky performance of $\geq 60\%$.

4.18 Baseline hematology and chemistry studies: hemoglobin > 9.9 gm/dl, total granulocyte count > 1,999/mm^3, platelet count > 124,000/mm^3, BUN < 30mg/dl, creatinine < 2mg/dl, alkaline phosphatase and SGOT < 2x normal upper limit of normal, amylase, lipase < 1.5x upper limit of normal. These studies must be performed within a week prior to starting therapy.

4.19 Informed Consent: All patients must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be performed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks and discomforts. Human protection committee approval of this protocol and consent form is required.

4.2 Registration

4.21 All patients must be enrolled onto study through the San Diego Regional Cancer Center prior to starting therapy. To register a patient, contact Colette Carson, R.N. , Clinical Trials Registrar (619-450-5990)
. Please provide the following information:

Physcian's name and phone number
Institution's name
Patient's name and hospital unit number
Patient's date of birth, sex, race, height and weight
Previous treatment (surgery, radiation therapy, chemotherapy) with dates
(must be > 6 weeks from enrollment or patient's tumor must be
progressing such that waiting 6 weeks is inadvisable)
Date and results of the following tests:
hemoglobin (must be > 9.9 mg/dL))
platelet count (must be > 124,000/mm^3)
BUN (must be < 30mg/dl)
creatinine (must be < 2mg/dl)
alkaline phosphatase, SGOT (must be < 2x normal upper limit)
amylase, lipase (must be < 1.5x normal upper limit)

4.22 The registrar will review the eligibilty checklist to verify patient eligibility. If the patient meets all criteria, the patient will be registered onto this study.

4.23 The registrar will remind the person registering the new patient of the expectations concerning submission of data and laboratory specimens.

5.0 Therapeutic Modalities

5.1 Preparation and Characterization of Biological Agents

5.11 Genetically Modified Tumor Cells

Tumor samples will be obtained from clinically indicated procedures under sterile conditions. The tumor tissue will be minced and placed in Richter's zinc option media to establish growth of tumor cells in culture. For some patients, the cultured cells will be genetically modified by retroviral mediated gene transfer to express the IL-2 gene product. Other patients will be treated with an HLA A2 positive allogeneic glioblastoma cell line transduced with the same IL-2 retroviral vector. All tissue processing procedures will be approved by the FDA.

5.12 Retroviral Vectors

The retroviral vector utilized in this study is derived from a murine retrovirus similar to the vectors employed by a number of investigators for *in vitro* and *in vivo* studies including recently approved investigations with human subjects (14). The cytokine vector used in this investigation (G1NaCvi2.23) was developed by investigators at Genetic Therapy Incorporated. This vector contains human IL-2 cDNA under the control of an internal CMV promoter.

Before utilization in human studies, the transduced cells will be certified to be free of contaminating replication competent retrovirus and other adventitious agents by criteria recommended by the FDA. The minimum assays to be performed will include the following:

Sterility testing by bacterial and fungal culture
Mycoplasma testing by Gen-Probe kit assay
Replication competent viral testing by feline PG-4 S+L- Assay

Additional assays will be performed as recommended by the FDA and outlined in Appendix 12.5.

5.13 Transduction Protocol

The cultured tumor cells will be incubated with conditioned supernatant from the producer cell culture transfected with the retroviral vector plasmids as described (15). The tumor cells will be washed and then grown in culture media containing G418, (a neomycin analogue) to select for transduced cells expressing the neo^R gene. G418-resistant cells will be stored at -70° C until required. Prior to immunization, the G418-resistant cells will be tested for expression of the IL-2 gene by measurements of the concentration of IL-2 in the culture supernatant by an enzyme-linked immunoabsorbent assay (ELISA).

5.14 Preparation of Irradiated Cells

After transduction and expansion, the genetically modified cells will be carried in continuous culture until there are sufficient cells for therapeutic applications and cyropreservation. The cyropreserved cells will be centrifuged and washed in Richter's zinc option media and then cryopreserved in a solution containing 10% dimethyl sulphoxide and 50% fetal calf serum. The cells will be stored in liquid nitrogen until the time of administration. Prior to their use in subcutaneous immunizations, the cells will be irradiated and resuspended in lactated Ringer's solution prior to injection. The transduced tumor cells will be treated with 7,000 rads prior to their use in immunizations. This dose of radiation treatment to the glioblastoma tumor cells was based on discussions with Dr. Herman Suit, Chief of Radiation Oncology at Massachusetts General Hospital. Dr. Suit indicated that based on data generated in his laboratory with 22 glioma cell lines, 7,000 rads would be the lowest radiation treatment sufficient to render the planned tumor cell doses incapable of proliferation and tumor formation. This dose was calculated from the following formula:

TCDp=D0[LnM+Lnn-(Ln-Lnp)]; where D0=reciprocal of terminal slope of cell survival curve (D0=290 rads for the most resistant cell line tested); TCDp=the dose which inactivates all cells at probability p=99; M=the number of cells to be treated; and n=extrapolation number (n=10 for the most resistant cell line tested). It is our desire to utilize the lowest possible radiation dose for the transduced cells to optimize the level and duration of IL-2 secretion. We have evaluated the effects of the proposed 7000 cGy dose on the GT9 cell line to be utilized in this study. This dose of radiation prevented the proliferation GT9 cells without inhibiting IL-2 secretion. The results of these studies are presented in more detail in Appendix 12.7 (Pre-Clnical Studies).

5.2 Potential Toxicity

Local toxicity at the sites of immunization are not expected to occur. If severe inflammation occurs at the site of injection, this will be treated by either topical steroid therapy and/or surgical excision of the inflamed tissues as indicated.

Hypersensitivity reactions are unlikely to occur, since the injected material is comprised of minimally modified cells. Systemic toxicity related to IL-2 administration should not occur as the low levels of IL-2 secreted by the genetically modified cells should not significantly effect systemic IL-2 concentrations.

There are several theoretical toxicities related to the use of retroviral mediated gene transfer. These theoretical toxicities are not expected to occur as the retrovirus utilized for gene transfer has been modified to prevent these toxicities. The retroviral vector has been altered to prevent viral replication by the deletion of viral gag, pol and env genes. Hence, the IL-2 retroviral vector is replication defective and patients' cells are never exposed to a replication competent virus. Replication competent retrovirus may theoretically develop by recombination between the IL-2 vector and viral gene sequences in the packaging cell line utilized to produce the retroviral vector. This type of recombination has rarely been

observed after extended culture. Hence, all retroviral vector supernatants used to infect patient cells will be screened for replication competent virus by standard assays (14). It is theoretically possible for the retroviral vector to insert the transgene into a proto-oncogene or tumor suppressor gene resulting in cellular transformation by insertional mutagenesis. The likelihood of this occurring is extremely small.

The neo^R gene transferred into the genetically modified tumor cells inactivates neomycin and related antibiotics such as amikacin. As the genetically modified cells will be injected subcutaneously, local expression of the neo^R gene is unlikely to effect treatment with these antibiotics at other sites. In addition, other more commonly used antibiotics such as gentamycin and tobramycin are not inactivated by the neo^R gene. Hence, the introduction of this gene into a local area of the patient is not expected to affect clinical management of infections.

5.3 Treatment of Toxicity

Local toxicity at the sites of cell administration will be treated with either topical steroids and/or surgical excision of the injection site as deemed appropriate. Hypersensitivity reactions may occur. Chills, fever and/or rash may be treated symptomatically with antipyretics and antihistamines. Patients should not be treated prophylactically.

Should arthralgias, lymphadenopathy or renal dysfunction occur, the investigators should be notified and treatment with corticosteroids and/or antihistamines may be instituted. Anaphylactoid type hypersensitivity reactions are not anticipated. However, should anaphylaxis occur, administration of epinephrine, fluids, steroids and cardiopulmonary support should be instituted as needed.

6.0 Treatment Plan

6.1 Location

Patients will be treated at Sharp Memorial Hospital, San Diego, CA.

6.2 Administration of Biological Agents

Patients will be randomly assigned to receive either autologous or allogeneic IL-2 transduced tumor cells. Subjects will receive at least three subcutaneous immunizations at least two weeks apart with one of the following preparations:

1) Irradiated autologous tumor cells genetically modified to secrete IL-2.
2) Irradiated allogeneic HLA- A2 positive GT9 tumor cells genetically modified to secrete IL-2.

All subjects will be treated as outpatients. Vital signs will be checked prior to immunization. Subsequently, vital signs should be obtained qlh x 2. The patients should be examined qlh x 2 for inflammation at the injection site and for evidence of rash, wheezing or edema. Provided that there are no contraindications, subjects may be discharged 2 hours after drug administration. If discharge is contraindicated, patients will be followed in the hospital on a regular basis as deemed appropriate.

7.0 Therapy Modification

7.1 Dose Escalation

We plan to initiate therapy with transduced cells that secrete 25 units of IL-2/24 hrs. This dose of IL-2. was well tolerated in our first patient and was the lowest dose associated with the generation of a cellular anti-tumor immune response. Our data indicate that the transduced cells will express approximatley 20

to 40 units of IL-2 per 10^6 cells per 24 hours. This will result in the administration of approximately 1.25 x 10^6 transduced cells for the initial immunizations depending upon the level of IL-2 expression measured *in vitro* per 10^6 cells over 24 hours. We plan to escalate the dose of IL-2 secreting cells, as follows:

Cohort	Number of Patients	Units of IL-2	# Transduced Cells
1	3	25	1.25×10^6
2	3	50	2.5×10^6
3	3	100	5.0×10^6
4	3	200	1.0×10^7
5	3	400	2.0×10^7

The transduced cells will be mixed with unmodified irradiated tumor cells resulting in a total tumor cell dose of 1×10^7 cells. The dose of transduced cells will be escalated when 3 patients at each dosage level have been treated and followed for 1 month without $\geq$ grade 3 toxicity. Toxicity grades are listed in Appendix 12.1 of the original protocol. Hence, we plan to study 15 patients with autologous transduced tumor injections and 15 patients with injections of the transduced allogeneic glioblastoma cell line GT9. If 3 patients at the same dosage level develop unacceptable treatment related toxicity ($\geq$ grade 3 toxicity), the study will continue at the previous dose level until three additional patients have been treated. The treatment of additional patients at the lower dosage level will permit further assessment of the effects of transduced cell injections at the lower dose. These parameters define the Phase I study.

However, if significant anti-tumor effects are observed in a given patient (>50% tumor reduction) it may be improper to withold further treatment if additional cells are available for immunization. These additional immunizations would be administered at 2 week intervals, at the discretion of the investigators, as warranted by the demonstration of anti-tumor immune responses and improvement in the patient's clinic status.

7.2 Maximum Tolerated Dose (MTD)

The MTD is defined as that dose of IL-2 secreting cells which results in $\geq$ grade 3 toxicity or which causes a delay in day 14 immunization in $\geq$ 3/6 patients in any treatment cohort. Day 14 treatments must be delayed if any $\geq$ grade 2 toxicity has not reversed to $\leq$ grade 1.

8.0 Study Parameters

8.1 Pre-treatment Evaluations

The following pre-treatment evaluations will be performed (8.11-8.14):

8.1.1 History and Physical Examination

These evaluations will include a description and quantitation of disease activity.

8.1.2 Performance Status Assessment

See Appendix 12.2 for Karnofsky Performance Status assessment.

8.1.3 Pre-treatment Laboratory

CBC with differential, platelet count, PT, PTT, glucose, BUN, creatinine, electrolytes, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, uric acid, calcium, total protein and albumin.

Urinalysis

CH_{50}, C3 and C4 serum complement levels

Immunophenotyping of peripheral blood B cell and T cell subsets (if sufficient cells are available)

PCR of peripheral blood leukocytes for neo^R and viral env

Serum IL-2 concentration

Humoral tumor immunity - serum anti-tumor titers

Cellular tumor immunity - Cytotoxic T cell assays against autologous tumor cells. Delayed type-hypersensitivity (DTH) skin testing will be performed at the discretion of the investigators and depending upon the availability of tumor cells. Aliquots of 10^6 autologous tumor cells will be utilized for DTH skin testing. The cells will be drawn up in 0.2 ml of normal saline and injected intradermally. Induration will be measured at 24 and 28 hours. A medium ball point pen will be placed at 45^o angle to the patient's skin, and the injection site approached from each of 4 sides until resistance is encountered. The area thus circumscribed will be measured in 2 diameters with a caliper. Selected DTH sites may be biopsied for histological and immunohistochemical analyses to identify the nature of infiltrating immune effector cell populations.

8.1.4 Other Pretreatment Evaluations

Chest X-ray and other diagnostic studies including computerized tomography (CT), magnetic resonance imaging (MRI) or radionuclide scans will be performed to document and quantitate the extent of disease activity.

8.2 Follow-up Procedures

After each immunization, patients will be contacted daily for 3 days to determine if the injection site is swollen or red. If a history of any erythema or induration is elicited, then the patient will be examined within 24 hours. Otherwise, subjects will be examined one week after the first immunization, then at approximately 1 to 2 week intervals. At each visit, the patients will be advised to return to the hospital and to contact one of the study investigators should they develop inflammation at injection sites or experience fever, rash, arthralgias, edema or dyspnea.

At each visit, the pertinent medical history will be obtained and a physical examination will be performed. Laboratory tests described in Section 8.1 will be performed at the weekly and monthly visits.

Diagnostic studies including X-rays, CT, MRI or radionuclide scans will be performed to evaluate responses to therapy.

Should toxicity occur, appropriate therapeutic intervention will be instituted. Patients will be followed until toxicity resolves.

A summary of the treatment plan including the schedule for drug administration and required monitoring evaluations in provided in Section 3.0.

8.3 Off-Study Criteria

8.3.1 A patient will be considered off treatment once protocol therapy has been stopped. Reasons for discontinuation of treatment include:

Development of grade ≥3 toxicity
Patient's tumor fails to respond to treatment (progressive disease)
Patient has completed therapy
Patient refuses further therapy
Patient develops other medical problems unrelated to the study that preclude further therapy.

8.3.2 A patient will be considered off study in the event of disease progression, death or initiation of non-protocol treatment.

8.3.3 A patient who is off study will be off treatment; however, a patient who is off treatment is not necessarily off study.

8.3.4 Patients who are off treatment but not off study will continue to be followed for diseaseprogression/response, late reports of toxicity and survival. Once a patient is off study because of disease progression or initiation of non-protocol treatment, the patient will be followed for survival purposes.

9.0 Evaluation Criteria

9.1 Adverse Drug Reaction Reporting

9.1.1 Reporting Requirements

The following adverse reactions must be reported to the study chair and the NCI, in addition, the institutions' IRB (Investigational Review Board) must be notified.

9.1.2 All life-threatening and lethal (grade 4 and 5) toxicity.

9.1.3 Grade 2 and grade 3 toxicity.

9.2 Types of Reports

9.2.1 Telephone Report. All life-threatening and lethal (grade 4 and 5) toxicity must be reported to IDB (301-230-2330) and the study chair by telephone within 24 hours.

9.2.2 Written Report. An original NCI Adverse Drug Reaction form and 2 copies must be submitted to the study chair within 5 days. Immediately upon receipt the chair will forward the original report to NCI.

9.3 Response Criteria

9.3.1 Following the first three cycles of treatment, an evaluation of response will be performed. Tumor size will be calculated by measuring the tumor's largest cross sectional area as measured by the

greatest cross sectional diameter times the greatest diameter perpendicular to it on enhanced CT or MRI. Technical parameters for the evaluation scan will be identical to those used for the baseline scan.

MRI methods routinely utilized in brain tumor clinical trials will be employed in our study. We are aware of the importance of employing uniform procedures for the performance and evaluation of MRI scans in monitoring tumor responses to therapy. A sagittal T1 weighted sequence will be performed initially using a TR of 600 and a 20 msec echo. An axial spin echo sequence with flow compensation will then be performed using a TR of 3000 with 30 and 80 msec echoes. Gadolinium will then be injected intravenously and contiguous 5 mm axial T1 weighted scans will then be performed through the entire brain using a TR of 800 and a 30 msec echo. Post contrast coronal T1 weighted scans will then be performed using a TR of 800 and a 27 msec echo. Tumor responses will be evaluated by comparing the tumor's largest cross sectional area as measured by the greatest cross sectional diameter times the greatest diameter perpendicular to it on enhanced MRI. Technical parameters for the evaluation scan will be identical to those used for the baseline scan. All of the MRI scans will be evaluated and performed under the direction of Dr. Keith Kortman, an experienced neuroradiologist.

9.3.2 A neurological examination will be performed and compared with the pretreatment examination.

9.33 A Karnofsky performance will be recorded and compared with the pretrement score.

9.34 Medications and their dosages, including steroids, and laboratory data will be recorded in accordance with the study parameters above.

9.35 Response will be defined by the criteria of MacDonald as summarized below.

Complete Response (CR): complete disappearance of all enhancing tumor on consecutive CT or MRI scans, off steroids and neurologically stable or improved.

Partial Response (PR): 50% or more reduction of contrast enhancing tumor area, steroid dose stable or reduced and neurologically stable or improved.

Progressive Disease (PD): 25% or more increase in contrast enhancing tumor area or new tumor focus.

Stable Disease (SD): All other situations.

9.36 CR, SD and PR will be considered responders; PD, and patients not evaluated after 2 cycles of treatment will be considered non-responders.

10.0 Data Submission

The following forms must be submitted to the study chair according to the following schedule:

Forms	Schedule
N-001 On-study form	Within one week of registration
N-002 Neuropathology Form	
N-004 Flow Sheets	

N-003 Follow-up Form N-004 Flow Sheet N-005 Chemotherapy Form N-006 Neuroradiology Form N-007 Toxicity Form	Every month while receiving protocol treatment and at completion of protocol treatment, then every three months until disease progrssion or initiation of alternative treatment
N-008 Death Form	At death
Adverse Reaction Reporting Form	Within five days for reportable toxic events

11.0 BIBLIOGRAPHY

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CONSENT TO ACT AS A RESEARCH SUBJECT

Injection of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL-2): A Phase I Study

I am being asked to participate in a research study. However, before I give my consent to volunteer, it is important that I read the following materials and ask as many questions as necessary to be sure that I understand what my participation will involve.

Investigator and Sponsor

Dr. Robert E. Sobol and his colleagues are conducting an experimental study to learn more about methods to transfer genes into cancer patients' cells that may enhance the body's immune responses against cancer. I have been asked to take part in this study because I have a glioblastoma, a form of brain cancer.

Purpose Of The Study

The purpose of this study is to determine the safety of injecting patients with genetically modified tumor cells. Tumor cells will be altered in the laboratory by having a new gene inserted to generate a substance, termed interleukin-2, which has been shown to stimulate anti-tumor immune responses in some cancer patients.

What Is Experimental About This Study

The process of injecting cancer patients with genetically modified tumor cells is experimental.

Number of Subjects

Approximately 30 patients will be treated.

Duration Of Study and Procedures To Be Followed

If I agree to be in this study, the following will happen to me: Either my own or another patient's genetically modified tumor cells will be injected underneath my skin. The tumor cells will have been treated with x-rays to prevent their ability to grow. These injections are similar in nature to those utilized for routine vaccinations. I will receive three of these injections at two week intervals. All injections will be administered to me as an outpatient. Following each injection, I will be observed for one hour to identify any side-effects of treatment. Subsequently, I will be contacted daily for three days following each injection and I will come to the clinic to be examined if redness or swelling develops at the injection sites. I will also be examined every two weeks during the first 8 weeks of the study, once a month for three months and every 3 months for one year after the completion of therapy and at least yearly thereafter to identify any side-effects of treatment and to evaluate the effects of these injections on

my cancer. Follow-up studies will also include blood tests at each visit. Approximately 100 cc (5-10 tablespoons) of blood will be obtained at these visits. Radiology studies such as x-rays or CT scans will be performed after the third injection.

Men and Women Who Can Have Children

I understand that both men and women who participate in this study must agree to to use an effective contraception method (such as condoms, the pill, diaphragm or intrauterine device) to prevent reproduction for the duration of the experimental treatment.

Because this is a new medication and it has not been given to pregnant women, it is not known how it would affect the unborn baby. If I am pregnant, breast feeding or think I might become pregnant, I can't be in this research study. If I am not pregnant and if I am using a method of birth control approved by my doctor, I can be in this research study. If I cannot have children because of menopause or surgical sterilization, I can be in the research study.

Medications

Before taking any drug other than the study injections, I should first discuss it with the study physician. This includes over-the-counter medications such as pain relievers and antacids.

I will tell my study physician or research assistant about the medications I am taking, including why and how often I take the medicine, and the strength of these medications.

I understand that I should not drive or operate complicated machinery if drowsiness or sedation results from any of my treatments.

Risks And Discomforts

Participation in this study may involve some added risks or discomforts. These include:

1. There may be pain, itching or a rash at the injection sites. These complications will be treated with medications, should they occur. If these side-effects at the injection sites are severe and do not respond to medications it may be necessary to surgically remove the affected tissues at the injection sites. Surgical removal of this tissue may be associated with pain, bleeding, infection and scar formation.

2. I may experience an allergic reaction to the injected materials. Allergic reactions may include symptoms such as fever, chills, rash, itching and low blood pressure. In some patients, these allergic reactions may be so severe as to be life-threatening. These allergic reactions will be treated in the event that they develop. Because this is a new and untried treatment, it is possible that other risks not mentioned and not presently known could occur.

3. The injections may induce immune responses against normal cells in the brain and cause encephalitis. Immune responses directed against normal tissues are termed "autoimmune reactions".

Other possible autoimmune reactions may include skin rashes and joint inflammation. These reactions will be treated with appropriate medications.

4. The research methods employed in this study utilize a virus to infect and transfer the interleukin-2 gene into the cells. No side-effects have been observed in limited human studies with this procedure. There are, however, some theoretical risks associated with these methods. While the virus utilized to transfer genes into my cells has been modified to prevent its ability to proliferate and infect other cells, it is theoretically possible for the virus to become infectious. Infectious viruses of this type have produced cancer in animals. A blood test will be performed to determine whether an infectious virus has been generated. If an infectious virus is detected, I agree to be isolated in a hospital room or my home until the virus disappears from my blood to minimize the risks of infecting other individuals.

5. The genetically modified cells will also contain a gene that produces a protein that inactivates certain antibiotics. These antibiotics are not commonly used in the treatment of human infections. In addition, as many other more commonly used antibiotics are not inactivated by this substance, the procedure is not expected to affect the therapy of bacterial infections that may occur during the course of my illness.

6. There may be some pain, bleeding, bruising or infection at the blood withdrawal sites in my arms. These potential complications are rarely severe.

Benefits Of The Study

There may be no direct benefit to me from this therapy. It is possible that these treatments may enhance my body's immune response to my cancer. However, even if this occurs, this may have no beneficial effect on the course of my illness. However, from my participation in this study, the investigators may learn more about the role of the body's immune responses against cancer and about the use of gene transfer methods. This information may prove useful in the therapy of cancer patients in the future.

Request for Autopsy

I have been informed that important information may be obtained from an autopsy in the event of my death at any time in the future. I give my permission for this evaluation.

Alternative Methods of Treatment

I understand that alternative experimental treatments may also be available or that I may be treated by supportive care only without experimental therapy.

Emergency, Side Effects, Illness or Questions About The Study

If I need medical treatment for any reason, I must contact my regular cancer physician or the study physician at the following telephone numbers:

Regular Physician/Phone:_____

Study Physician: San Diego Regional Cancer Center: 619-450-5990

If I have any questions about this study, please call: Dr. Robert E. Sobol at 619-450-5990 between the hours of 8am - 4pm.

Compensation for Injury

The cost of treatment for injuries that result from participation in this research will be covered. I understand that no other form of compensation will be provided if I am injured. For further information about this, I may call the Human Subjects Committee Office at 619-450-5990.

Confidentiality

My research records will be kept confidential, but will be available for examination by qualified representatives of the National Institutes of Health, Food and Drug Administration, manufacturers of the preparations utilized in the study, and hospital personnel.

Subject Cost/Payment For Participation

There will be no additional charges for my participation in the study. I will not receive any money for participating in this study.

Subject Rights And Study Withdrawal

Participation in research is entirely voluntary. I may refuse to participate or withdraw at any time without jeopardizing my future healthcare benefits. I understand that I am not giving up any of my legal rights by signing this consent form.

Participation in this study may be ended without my consent if:

- the investigator believes that it is in my best interest.
- I don't follow the study procedures.
- the sponsor stops the study or tells my doctor that I must stop the study.

It is important that I understand all of the important details about the study. If I have any questions that are not answered by these materials, I should contact the study physician for more information.

If I have any questions about my rights as a subject in a research study, I should contact the Institutional Review Committee Chairman through

Linda Bergen, Coordinator
Institutional Review Committee
(619) 450-5990

This research project has been reviewed by an institutional review board for the protection of the rights of human subjects in research studies, in accordance with federal regulations.

Signature And Acknowledgment

My signature below means that I have read the above information about **Injection of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL-2): A Phase I Study** and I have had a chance to ask questions to help me understand what I will do in this study. I agree to be in the study and I have been told that I can withdraw from the study at any time. I have been given a copy of this agreement and a copy of the Experimental Subject's Bill of Rights. I have been told that by signing this consent form I am not giving up any of my legal rights.

_____ _____
SIGNATURE OF SUBJECT DATE

_____ _____
SIGNATURE OF WITNESS DATE

_____ _____
PHYSICIAN'S SIGNATURE DATE

1/94

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME	POSITION TITLE
Robert E. Sobol, M.D.	Adjunct Associate Member

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Boston University, Boston, MA	B.A.	1974	Philosophy
Chicago Medical School, Chicago, IL	M.D.	1979	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1979-1980	Internship, Internal Med, University of Southern California Medical Center, Los Angeles, California
1980-1982	Research Fellow in Hematology/Oncology, University of California, San Diego, San Diego, California
1982-1983	Assistant Research Immunologist, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1983-1990	Associate Physician, University of California, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1986-1989	Resident, Internal Medicine, Mercy Hospital, San Diego, California
1990-1992	Clinical Fellow in Hematology/Oncology, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1991-Present	Adjunct Associate Member, San Diego Regional Cancer Center

Publications (Partial list of 65 publications)

1. Sobol RE, Dillman RO, Smith JD, et al: Phase I evaluation of murine monoclonal anti-melanoma antibody in man: Preliminary observations. Hybridomas in Cancer Diagnosis and Treatment. Mitchell MS, Oettgen HF (eds). Raven Press, New York, Prog Cancer Research Thor 21:199-206, 1982.
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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Ivor Royston, M.D.	President and Scientific Director

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
George Washington Univ., Washington, DC			Pre-medicine
Johns Hopkins Univ., Baltimore, MD	B.A.	1967	Human Biology
Johns Hopkins Univ., Baltimore, MD	M.D.	1970	Medicine
Stanford University, Stanford, CA		1977	Medical Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1970-71 Intern, Internal Medicine, Stanford University Hospital, Stanford, CA
1971-72 Resident in Internal Medicine, Stanford University Hospital, Stanford, CA
1972-73 Staff Associate, Division of Virology, Bureau of Biologics, NIH, Bethesda, MD
1973-75 Chief, Viral Oncology Section, Division of Virology, Bureau of Biologics, NIH, Bethesda, MD
1975-77 Post-doctoral Fellow, Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA
1977-78 Staff Physician, Veterans Administration Medical Center, San Diego, CA
1977-82 Assistant Professor of Medicine, Division of Hematology/Oncology, University of California, San Diego, School of Medicine, La Jolla, CA
1978-81 Clinical Investigator, Veterans Administration Medical Center, San Diego, CA
1980-91 Immunology & Genetics Committee, Cancer and Leukemia Group B
1981-91 Vice Chairman, Immunology and Genetics Committee of Cancer and Leukemia Group B
1981-90 Director, Cell Surface Marker Laboratory, UCSD Cancer Center, San Diego
1981-84 Chief, Medical Oncology, San Diego Veterans Administration Medical Center, San Diego, CA
1982-90 Associate Professor of Medicine, Division of Hematology/Oncology, UCSD School of Medicine
1982-85 Consulting member of the Biological Response Modifiers Program Decision Network Committee, Division of Cancer Treatment, National Cancer Institute/National Institutes of Health
1983-88 Member, Clinical Cancer Program Project Review Committee, NCI/NIH
1984-90 Director, Clinical Immunology Program, UCSD Cancer Center
1986-88 Member, UCSD Biotechnology Transfer Advisory Committee
1986-Pres. Co-Director, International Conference on Monoclonal Antibody Immunoconjugates for Cancer
1989-90 Member, Long Range Planning Committee, UCSD Cancer Center
1990-Pres. President & Scientific Director, San Diego Regional Cancer Center, San Diego, CA
1990-Pres. Adjunct Professor of Medicine, UCSD School of Medicine, San Diego, CA
1992-Pres. Co-Director, International Gene Therapy of Cancer Conference
1992-Pres. Member, Department of Veterans Affairs Merit Review Board for Oncology
1992-Pres. Member, Molecular Biology of Cancer Editorial Board
1992-Pres. Member, American Association for Cancer Research Exhibits Committee
1992-Pres. Chairman, Special Review Committee-Clinical Trials of Cancer with BRMs, NCI-NIH
1993-Pres. Member, Biotechnology Advisory Committee, for Congresswoman Lynn Schenk

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HUMAN GENE TRANSFER PROTOCOL

9406-081 Lotze, Michael T; University of Pittsburgh, Pittsburgh, Pennsylvania; *IL-12 Gene Therapy Using Direct Injection of Tumor with Genetically Engineered Autologous Fibroblasts.*

Date of RAC Approval: 6/10/1994

This protocol studies patients with advanced cancer who have failed standard therapy. The major goal is to treat cutaneous lesions and to enhance the patient's immune reactivity to their own tumor. The interleukin-12 (IL-12) subunit genes (p35 and p40) and a selectable marker, neomycin phosphotransferase, will be introduced into a fibroblast line established from the patient using a retroviral vector. These gene-modified fibroblasts will then be injected peritumorally into the patient. This injection may have direct antitumor effect. It may also augment the immune response of the patient since the immune stimulatory effects of the IL-12 secreted by the gene modified fibroblasts may be systemic. To further evaluate the immune response of the patient to their own tumor, irradiated tumor cells or common recall antigens will be inoculated to patients as skin tests. Animal models have shown that injection of gene modified fibroblasts has important antitumor effects. A total of four injections will be performed in four weeks.

The patients will be evaluated for antitumor effects engendered by the peritumoral injection of the gene modified fibroblasts. The injection of gene modified fibroblasts may serve to "immunize" the patient to their tumor and may be amenable for use in a wide variety of tumor types, especially those that are poorly immunogenic. This protocol may also increase the effectiveness of active immunotherapy as well as expand the use of subsequent administration of IL-2 to patients with other malignancies not currently amenable to immunotherapy.

NONTECHNICAL ABSTRACT OF PROTOCOL

Non-Technical Abstract

Many patients have advanced cancer that does not respond to standard therapies. For these patients, a new approach to treat their cancer (tumor) will be tested. Animal studies have been done using either cancer cells or their own skin fibroblast cells into which a cancer fighting gene has been placed. When these modified cells are returned to the animal the animal can "learn" to fight the cancer cells better. The return of the gene modified cells can be helpful in fighting cancer in several ways.

1. Injecting the patient's own skin cells (fibroblasts) right next to the tumor has been shown to reduce the size of tumor when these cells were genetically engineered to express cancer fighting genes. At the same time, this treatment has been shown to increase the responses of the animal's white cells in fighting the tumor cells as well.
2. Before returning fibroblasts to the patients, they will be altered to release a potent white cell activating factor (interleukin 12) by placing both the genes required to produce interleukin-12 into them. If these gene modified cells are used, the release of interleukin-12 by fibroblasts will increase the ability of the animal's white cells to fight cancer cells by activating the patient's own white cells.

This protocol is designed to mimic the results obtained with animals.
Patients with cancer who have not responded to other therapy will have a piece of skin cut out and brought to the laboratory. In the laboratory, the interleukin-12 gene will be put into the patient's normal cells (fibroblasts) grown from the skin. The fibroblasts are then injected right next to the tumors existing in the skin of the patient. This injection will be repeated once a week for four times in total. During this time and afterwards the patient is watched for signs that the therapy might be helpful in destroying their cancer. Since this therapy is new, it is not known how well it will work in people. The patients will also be watched for possible harmful effects from the therapy. After patients with several different kinds of cancer have received this new therapy, we will be able to determine how safe this new therapy is and begin to assess how well it works.

IL-12 Gene Therapy using direct injection of tumors with genetically engineered autologous fibroblasts

Principal Investigators:

Hideaki Tahara, M.D., Ph.D.
Michael T. Lotze, M.D.

Coprincipal investigators:

Paul D Robbins, Ph.D.
Walter J. Storkus, Ph.D.
Laurence Zitvogel, M.D.

Associate Investigators:

Surgical Oncology

Howard Edington, M.D.
Jeffrey Myers, M.D., Ph.D.
Chet Nastala, M.D.
Itzhak Pappo, M.D.
Joshua T. Rubin, M.D.
Herbert J. Zeh III, M.D.

Medical Oncology

John Kirkwood, M.D.
Theodore Logan, M.D.

Urology

Raoul R. Salup. M.D.,

Radiation Therapy

Maury Rosenstein, Ph.D., M.D.

Molecular Genetics and Biochemistry

Gunhild M. Mueller. Ph.D.
Joseph C. Glorioso, Ph.D.

Cell Biology and Physiology

Simon C. Watkins, Ph.D.

IMDL/CT

Theresa Whiteside, Ph.D.
Elaine Elder, Ph.D.

Human Gene Therapy Applications Laboratory (HGTAL)

John A. Barranger, M.D., Ph.D.
Alfred Bahnson, Ph.D.

Ethics

Rosa Lynn Pinkus, Ph.D.

Statistics

John Bryant, Ph.D.

Genetics Institute, Inc.

Stanley F. Wolf, Ph.D.

Hoffmann-La Roche Inc.

Maurice K. Gately, Ph.D.
Ueli Gubler, Ph.D.

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1.0 INTRODUCTION AND BACKGROUND

1.1 Need for novel therapeutics

Most patients with disseminated and/or locally far-advanced cancer can not be cured using conventional therapies including surgical resection, radiation therapy, and chemotherapy. Thus, it is apparent that additional therapeutic strategies are needed. We, and others, have previously demonstrated that interleukin-2 (IL-2)-based immunotherapy induces clinically significant tumor regression in 20 to 30 percent of treated patients who have metastatic melanoma or renal cell carcinoma, respectively (Rosenberg et al, 1989). Responses have been noted, albeit much less frequently, among treated patients who have other malignancies including colon carcinoma, ovarian carcinoma, breast carcinoma, lung cancer, and non-Hodgkin's lymphoma (Rubin et al, 1992). Although a small proportion of patients manifest durable clinical responses, prolonged survival, cure, or even palliation have yet to be clearly demonstrated.

To improve the clinical outcome of existing systemic cytokine therapies, alternative strategies for immunotherapy have been tested to show their efficiency in in vitro and in vivo tumor models. In such studies, we have been accumulating evidence which suggests that the cytokine microenvironment at the tumor site may determine, in part, the outcome of the immune response (Fearon et al, 1990. Gansbacher et al, 1990. Tepper et al, 1989).

1.2 Interleukin-12 (IL-12) Biology

IL-12, formerly termed natural-killer cell stimulatory factor (NKSF)(Kobayashi et al, 1989) or cytotoxic lymphocyte maturation factor (CLMF)(Stern et al, 1990), is a disulfide-linked heterodimeric cytokine composed of a 35 kD light chain (p35) and a 40 kD heavy chain (p40) (Kobayashi et al, 1989. Stern et al, 1990). The cDNAs encoding the p35 and p40 chains of IL-12 from both the mouse and human have been cloned. Unlike most other cytokines, simultaneous transfection of mammalian cells with two different genes is necessary for production of biologically active IL-12 (Wolf et al, 1991. Gubler, 1991). This cytokine exerts a variety of biological effects on human T and NK cells in vitro. These include the ability to synergize with IL-2 in augmenting allogeneic CTL response (Wong et al, 1988), LAK activity (Stern et al, 1990), and INF-g production from peripheral blood lymphocytes (Kobayashi et al, 1989. Chan et al, 1991). IL-12 also directly stimulates the production of IFN-g and other cytokines from peripheral blood T and NK cells (9,10), enhances the lytic activity of NK cells (Kobayashi et al, 1989; Naume et al, 1992), and promotes the expansion of activated NK cells and activated T cells (CD4+ and CD8+ subsets)(Gately et al, 1991). IL-12 has also been shown to induce primarily a T_H1 (cellular immune) response in vitro (Hsieh et al, 1993). In addition to the in vitro results, recent studies with the administration of recombinant murine IL-12 to normal mice revealed that IL-12 enhances NK and CTL activity and induces IFN-g production in vivo (Gately et al, 1993). Immunization with IL-12 in a murine Leishmania model suggests the role of IL-12 as a potent adjuvant (Afonso et al, 1994).

Based on these results which suggested profound immunologic effects of IL-12 alone or in combination with IL-2, we initiated murine therapeutic studies to investigate the possible anti-tumor effect of IL-12 (Tahara et al, 1994; Nastala et al, 1994). These experiments demonstrated that systemic application of IL-12 can suppress and prolong the survival of tumor bearing mice even when IL-12 treatment is started as late as 14 days after tumor

inoculation. Such therapeutic effects were also reported in similar models by Brunda, et al. (1993). In their studies, therapeutic intervention with systemic administration of IL-12 can be initiated as late as day 28 after injection of tumor cells (M5076 reticulum cell sarcoma) resulting in inhibition of tumor growth, reduction in the number of metastases and an increase in survival time. Although IL-12 has demonstrated potent anti-tumor effects when injected systemically, induction of long term immunity is less frequent and variable from experiment to experiment (Brunda et al, 1993. Nastala et al, 1994). Interestingly, Brunda et al observed that the best results with systemic IL-12 administration (complete regression of the tumor and induction of protective immunity against tumor re-challenge) was observed following peri-tumoral injections of IL-12 in a subcutaneous tumor model using Renca cells (Brunda et al, 1993). Since IL-12 is secreted only by "professional" antigen presenting cells (i.e. macrophages and B cells), it is possible that local administration of IL-12 at the site of tumor might approximate the role of IL-12 in eliciting an endogenous immune response. More recent evidence suggest that IL-12 plays a critical co-stimulatory role with B7 on APC in inducing proliferation and IFN-g production from T_H1 clones (G Trinchieri, personal communication). Thus, IL-12 may be the ideal cytokine for inducing tumor specific immunity when administered at the tumor site. Local secretion of the prototypic T-cell growth factors, IL-2 and IL-4 (Fearon et al, 1990. Gansbacher et al, 1990. Tepper et al, 1989), has been previously achieved through the introduction of genes directly into tumor cells. This strategy, however, has many obstacles precluding successful clinical application: tumor cells may be difficult to culture and transfect, and selection for transfected cells may require prolonged culture and alter expression of nominal tumor antigens. To avoid these potential disadvantages, we have developed an alternative approach to obtain paracrine cytokine secretion using fibroblasts genetically modified to secrete IL-12. The advantages of this strategy are that fibroblasts 1) can produce physiologically relevant levels of cytokine after transfection, 2) are readily available to culture, transfect and select, and 3) are less likely to migrate to other sites compared to tumor or lymphoid cells.

1.3 IL-12 Paracrine Delivery

To examine the effects of the rationale of IL-12 paracrine therapy described above, we have established animal models using fibroblasts genetically engineered to secrete murine IL-12 and examined its effects on tumor growth (Tahara et al, 1994). NIH3T3 cells were transfected with expression plasmids carrying both murine IL-12 genes, p35 and p40, using the calcium phosphate method (Sambrook et al, 1989) to express 100 - 240 Hoffmann-La Roche units/10^6 cells/48 hours of IL-12. IL-12 levels were measured in a 4 d. human PHA blast assay and confirmed to be IL-12 by a PHA blast assay performed on the protein "captured" in microtiter wells by IL-12 specific antibody. The effects of paracrine secretion of IL-12 on tumor establishment and vaccination models were examined using the poorly immunogenic murine melanoma cell line BL-6 in C57BL/6 (B6) mice. To determine the effects of IL-12 on tumor formation, non-irradiated BL-6 cells were subcutaneously inoculated into B6 mice admixed with NIH3T3 cells transfected with both subunits of mIL-12(3T3-IL-12) or with cells transfected with only the neomycin phosphotransferase gene (3T3-Neo). Compared to mice injected with BL-6 alone, the day of emergence of detectable tumors was significantly delayed in mice injected with BL6 admixed with 3T3-IL-12, but not in mice with BL6 admixed with 3T3-Neo (Fig. 1a). Effectiveness in this system was related to the amount of IL-12 expressed by the 3T3-IL-12 (Fig. 1b).

To determine whether locally secreted IL-12 at the tumor site induces

effective anti-tumor immunity, 10^6 irradiated tumor cells mixed with 3T3-IL-12 or 3T3-Neo were injected as a vaccine, and the response to a tumor challenge was subsequently examined (Fig. 2). With a tumor challenge of less than 1 x 10^5 non-irradiated BL-6 cells, significant delay of establishment of tumor was noted with a relatively small amount of IL-12 secretion (1.2 Hoffmann-La Roche units / 5 x 10^5 cells / 48 hours).

These results suggest that local delivery of IL-12 inhibits tumor growth in a dose dependent manner and leads to the development of an anti-tumor immune response.

1.4 Development of TFG-IL-12 Retroviral Vector (Murine and Human)

Our previous system uses allogeneic 3T3 cells as a means to continuously deliver IL-12 at the site of tumor. However, these cells probably induce an allogeneic immune response thus effectively eliminating long term IL-12 production. This makes direct interpretation of our results somewhat complicated. Development of efficient vectors for transfection into appropriate autologous cells will enable us to ask questions raised by our studies and also test more clinically relevant systems. Thus, we have constructed a retroviral vector which allows us to express genes of both subunits of mIL-12 with a selectable marker. The requirement for simultaneous expression of p35 and p40 for production of biologically active IL-12 (Wolf et al, 1991), presented unique problems in the creation of these vectors. The existing strategies using regulated splicing or heterogeneous promoters raised substantial concern about discordant production of the two chains from the transfected cells. To overcome this problem, we developed vectors utilizing an internal ribosome entry site (IRES) sequence. The IRES sequence used was obtained from the 5' nontranslated region of equine encephalomyocarditis virus (EMCV). Other picorna-viruses have similar sequences which allow cap-independent translation initiation from the internal ribosome entry site. A single polycistronic transcript from two separate genes can thus be transcribed following a single promoter and a second gene can be translated in a cap-independent manner. A modified MFG based retroviral vector (DFG-mIL-12, Fig. 3a) was constructed using an IRES fragment of EMCV, obtained from the pCITE plasmid (Novagen, Madison, WI). The resultant construct is shown in Fig.3a. DFG-human IL-12 has also been constructed in a similar manner to prepare for this clinical application (Zitovogel et al. 1994). To obtain a retrovirus which carries both the mIL-12 genes and the neomycin phosphotranspherase gene as a selectable marker, the IRES-Neo (pMC1neo Poly A, Strategene, La Jolla, CA) cassette was cloned into the site adjacent to the p35 cDNA in DFG-mIL-12 to create a vector capable of coordinately expressing three independent genes, termed TFG-mIL-12-Neo, Fig. 3b). Retroviral supernatant was generated using DFG-mIL-12 and the CRIP packaging cell line (Danoa et al, 1988) or using TFG-mIL-12-Neo and the BOSC23 packaging cell line (Pear et al, 1993). We now have over six months of experience with a stable producer cell line (ΨCRIP-TFG-IL-12-Neo).

1.5 Production of IL-12 Following Retriviral Transduction

Expression of these retroviral vectors was measured using NIH3T3 cells. These cells were infected with the supernatant of the producer cell line, and their supernatant was measured for expression of the transfected genes (Table 1).

Table 1 Expression of the transfected NIH-3T3 cells

Vector(s)	Transfection method	Selection	IL-12 secretion*
BL-pSV-p35 & p40	Ca-Ph.	+ (Colony #8)	2.1
DFG-mIL-12	Infection	-	5
TFG-mIL-12	Infection	+ (bulk)	120

*: Measured as (ng / 10^6 cells / 48 hours)

These results demonstrate the ability of our vectors to efficiently transfect and induce production of large quantities of biologically active IL-12 from transfected fibroblasts.

We used the TFG-mIL-12-Neo vector to genetically engineer fibroblasts which were injected directly into the subcutaneous tumor. Our results clearly showed that IL-12 not only has a profound effect on suppressing tumor growth, but also in promoting protective immunity to a subsequent tumor challenge. Thus, if local injection of fibroblasts expressing IL-12 can induce a well established tumor to regress and promote anti-tumor immunity at the same time, this strategy might be applicable in the clinical setting.

A preliminary experiment was done using infected 3T3 cells expressing high levels of IL-12 (3T3-TFG-IL-12: 120 ng / 10^6 cells / 48 hours). 3 x 10^5 MCA207 (murine methycholanthrene sarcoma) cells were inoculated intradermally into the left flank of C57BL/6 mice on day 0. Seven days after the inoculation, saline, 1 x 10^6 3T3-Neo, or 1 x 10^6 3T3-TFG-IL-12 were injected peri-tumorally into the well established tumor (mean size of tumors at the time of IL-12 treatment; 36 mm^2), and the tumor area was measured in a blinded fashion (Fig. 4). In the 3T3-Neo treatment group, 7/8 tumors grew progressively after injection. One of these animals exhibited rejection of their tumors following injection of the allogeneic 3T3 line, however it recurred. 8 of 9 tumors treated with 3T3-TFG-IL-12 transfected fibroblasts disappeared within 2 weeks after treatment. Three of these animals experienced recurrence of their tumors around day 25, but one of them dissappeared again. In summary, 6 of 9 animals with well established tumor were apparently cured (>37 days). These mice were challenged with 5 x 10^5 MCA-207 tumor cells in the other flank, and all of them rejected the subsequent challenge. A profound anti-tumor effect of local IL-12 delivery was observed in this experiment. This approach is appealing since large number of live tumor cells existing in the tumor nodule can be used as antigen in contrast to the existing gene therapy vaccination protocols previously approved.

To date, in vivo gene therapy models which demonstrate such anti-tumor effects have utilized only a few different strategies. These include in vivo gene transfer of the herpes simplex thymidine kinase (HSV-TK) gene using direct injection of the retroviral producer cell line (Culver et al, 1992) and one using a liposome-DNA conjugate (Nable et al, 1992). Although clinical application of our approach still uses a somewhat complicated procedure with propagation and transfection of autologous fibroblasts in culture, there are significant theoretical advantages compared with some of these other approaches. Our procedure 1) is independent of transfection efficiency which might be very different in vivo 2) can avoid delivery of infectious viral particles to the host, and 3) does not include potential immunogens other than the nominal tumor antigen. These hypothetical advantages provide an additional rationale for proceeding with this IL-12 gene therapy protocol.

2.0 OBJECTIVES

2.1 Define the local and systemic toxicity associated with peritumoral injection of fibroblasts genetically engineered to secrete IL-12
2.2 Examine the local and systemic immunmodulatory effects of peritumoral injection of fibroblasts genetically engineered to secrete IL-12 into human tumors.
2.3 Evaluate the clinical efficacy of this procedure.

3.0 PROTOCOL SCHEMA

Harvest Skin, Establish and Expand Fibroblast Line

Transduce and Expand
Insert IL-12 gene, select with G418, and expand

IL-12 Elisa and Bioassay
Activity in culture supernatant
when cell population reaches 1 X 10^7

Harvest
up to 5 X 10^7 Fibroblasts

Prepare Injection
5000 rads

First Injection-day 0
2 sites: Injection sites will be marked by Indian Ink
Treatment site:Fibroblasts transduced with the IL-12 genes
(Number of the fibroblasts will be adjusted to achieve the desired total amount of IL-12 secretion*)
Control site: Nontransduced fibroblasts
(The same number with transduced fibroblasts)

Second Injection-day 7
Same procedures as the first injection without control fibroblast injection
Injection sites will be examined at this visit

Third Injection-day 14
Same procedures as the second injection

Fourth Injection-day 21
Same procedures as the first injection

Physical Examination and Biopsy-day 28
One or multiple sites will undergo biopsy

Clinical and Radiographic Assessment-day 60 and 90

*: Total dose of IL-12 will be 10 ng/24 hours initially for the first three patients. After evaluation of adverse effect of initial three patients, the amount will be increased to 30 ng/24 hours. This sequence will be followed to 100, 300, 1,000, and 3,000 ng/24 hours. All three patients will need to be followed for at least two weeks after the previous dosage and judged to be without significant toxicity before escalating to the next dosage.

4.0 DESIGN OF CLINICAL PROCEDURES

4.1 Obtaining Fibroblasts for Use in this Study:

In order to obtain large numbers of proliferating cells that can be transduced and available for use in a short period of time, we propose to use cultured, autologous fibroblasts grown from the skin resected at the time of metastectomy or as an outpatient procedure alone (19.2). An elipse of skin measuring 10cm by 2cm will be resected from a convenient location such as the anterior abdominal wall. This will be performed in sterile fashion using local anesthesia. All wounds will be closed primarily with either sutures or staples. The skin will be transferred in sterile fashion to the laboratory where it will be cleared of fat, divided into 1mm-2mm diameter pieces incubated with tryple enzyme solution (Detailed protocol; Appendix 19.1) and plated in T25 flasks containing sterile culture medium and 10% human AB serum. Cultures will be split 1 to 3 when confluent monolayers develop. After the first passage, the fibroblasts will be transduced and replated as described below.

4.2 Transduction and Growth of Fibroblasts:

The procedures used here are the same as those used in our previous protocols at the NIH and the PCI, involving the infusion of TIL transduced with the Neo resistance gene (protocol 86-C-183c and 90-C-183c)(Rosenberg et al, 1990) and our IL-4 gene therapy protocol. These are detailed in the appendix (19.3). Cultured fibroblasts will be transduced during log phase of growth using a retroviral vector containing the genes for human IL-12 and neomycin phosphotransferase. Forty-eight hours after transduction, they will be exposed to the selective pressure of 0.1 - 1.0 mg/ml of G418. This concentration may be increased to as much as 1 mg/ml depending on the health of the culture. Quality control of the retroviral supernatant and transfected fibroblasts will be conducted strictly observing FDA regulation. These regulations include detection of replication competent retrovirus using detection of <u>env</u> protein of retrovirus and other potential pathogens including mycoplasma or other bacteria. This testing and the growth of the fibroblasts will be conducted by the Immunologic Monitoring and Diagnostic Laboratory (IMDL) of the PCI. GLP will be followed. Fibroblasts will be cultured in the IMDL.

4.3 Preparation of the IL-12 Vector-Containing Supernatant:

Unlike our previously approved protocol of IL-4 gene therapy (P/I: Michael T. Lotze and Joshua T. Rubin), supernatants for this clinical trial will be prepared in the Human Gene Therapy Applications Laboratory (HGTAL) at the University of Pittsburgh HGTAL using the retroviral constructs developed by our group (Zitvogel et al, 1994). Thus, certification of the retroviral construct and helper-free viral supernatant for human clinical trials will be conducted based on our experience in Gene therapy for Gaucher Disease (P.I. John Barranger). Testing for existence of helper virus in the viral supernatant and other adventitial agents will be carried out under contract with Microbilogical Associates (Appendix). The entire proviral construct, termed TFG-human IL-12-Neo, will be sequenced, and the packaging cell line, termed YCRIP, will be certified free of mycoplasma. This proviral construct will be tranfected into this certified packaging cell line using standard calcium phosphate methods to obtain retroviral supernatant. They will be tested and shown to be free of adventitious agents including RCR (replication competent retroviruses) replication-competent virus using the NIH 3T3 and S+/L- assays.

4.4 Preparation and Injection of Fibroblasts.

When the total population of fibroblasts reaches about 1 X 10^7 cells, the medium will be changed and the amount of IL-12 appearing in the culture supernatant of representative flasks over 24 hours will be determined by ELISA for the heterodimeric human IL-12 and in a bioassay. This will be used to estimate the amount of IL-12 that may be elaborated at each injection site per 10^7 cells per 24 hours. Fibroblasts will then be harvested by brief trypsinization and prepared for injection with diluted in appropriate amount of saline.

Accessible cutaneous tumor sites will be measured and recorded. If there is only one or two cutaneous lesions accessible in patient's skin, the injection sites will be restricted to the circumference of the tumor so that control fibroblasts are injected at 12 0' clock and 1/3 of the preparation will be injected at 1, 5, and 9 0' clock. Similarly, the second injection will be at 2, 6, and 10 0'clock as described in Fig. 5. Subsequently, injection sites will be marked with India ink. Injection sites can be identified and evaluated after the treatment. If there are many (more than four) accessible cutaneous lesions which are smaller than 1 cm in diameters, one or two lesions could treated in each injection day.

Patients will first undergo primary peritumoral injection at tumor sites using transduced fibroblasts or non-transduced fibroblasts at a single control site. A preparation of fibroblasts will be suspended in 0.1 to 1 ml of sterile saline depending on the tumor size and amount of fibroblasts. The cells will be irradiated with 5,000 rads prior to injection. At the time of administration, a small aliquot of fibroblasts will be set aside to test for lack of growth and for production of IL-12 following irradiation.

The number of transduced fibroblasts to be injected will be determined by the estimated amount of IL-12 secreted from them based on the results of *in vitro* assay. The estimated amount of secreted IL-12 will be 10 ng/24 hours in total for each weekly injection for the first three patients. The value of 10 ng/24 hours is the achievable amount of IL-12 secreted from 1 x 10^6 fibroblasts transfected with our current vector system and about 1/5 of the dose used in our day 7 established murine tumor models. This amount is about 1/70 of the amount of the proposed initiating dose in phase I clinical trial of intravenous injection of recombinant human IL-12 (10 ng/kg), and it is 1/350,000 of the maximal tolerated dose in primate models when given systemically. Thus, we consider this dose reasonable and safe as an initiating value. After evaluation of adverse effects, the amount will be increased to 30 ng after at least two weeks of observation of the initial three patients. This sequence will be followed by 100, 300, 1,000 and 3,000 ng/24 hours after the completion of the evaluation of three patients at each dose level.

Each week after injection, the size and characteristics of the injected tumors will be measured in two diagonal diameters and recorded. This injection will be repeated provided that the patient has not suffered a severe local reaction such as abscess or ulceration. The same dose of IL-12 producing fibroblasts per tumor will be injected, each week for a total of four weeks. The local level of IL-12 in regressing lesions has not been directly measured. Although direct determination of this value is difficult, previous study of IL-4 transfected fibroblasts in clinical trials of IL-4 gene therapy showed that mRNA expression of transfected IL-4 can be detectable up to seven days with a gradual decrease over this time period. It is a general understanding that *in vivo* expression of the transfected gene is less than that observed *in vitro*. Thus the amount of IL-12 secretion in the regressing tumor lesions should be smaller than the calculated amount based on the results *in vitro* which would be estimated as 130 ng/48 hours.

Four weeks after the first injection, tumor size will be examined and recorded. After recording, one of the injected tumors will be excisionally biopcied if possible and evaluated to determine the direct antitumor effect of the injection and the dose of IL-12 associated with the most intense mononuclear cell infiltrate. If not possible to excise, 6 mm punch biopsies of the sites indicated by India ink will be performed.

4.5 Preparation Testing:

Prior to administration of the transduced fibroblast preparation, the following evaluations will be performed:

A. Quantitation of IL-12 secretion by transfected fibroblasts- A minimum of 0.5 ng/ 10^6 cells /24 hrs and no more than 1000 ng / 10^6 cells /24 hrs of IL-12 by transfected fibroblasts will be used as criteria for treatment. IL-12 expression from the transfected fibroblasts is predicted to be far less than 2 mg/day total (0.04 mg/kg/day), and Dose of up to 50 mg/kg/day have been well tolerated (see 10.1) (Data on file, Genetics Institute) .

B. Microbiology testing for sterility- After each split of the fibroblasts (approximately once a week), cultures will be sent to the microbiology laboratory to detect possible contamination with bacterial organisms. Mycoplasma testing will be performed one week before infusion by a commercial testing service.

C. S+/L- assay including 3T3 amplification will be performed following injection of transduced fibroblasts to detect replication competent retrovirus.

4.6 Immunologic Monitoring:

Fifty ml of blood will be collected in four green top tubes and 1 red top tube on days 0, 7, 14, 21, and 28. Peripheral blood mononuclear cells will be cryopreserved at -180° C and serum will be frozen at -20°C. These specimens will be used to detect alterations in the phenotype of circulating lymphocytes and to quantitate systemic IL-12 and INF-g levels, respectively. Patients will be evaluated daily in the clinic for 1 week after each vaccine is administered. The sites will be assessed for the presense of induration, erythema, tenderness, ulceration, or infection. Patients' temperatures and vital signs will also be monitored. The area of erythema and induration will be measured. Injection sites will be excised as described above under local anesthesia in sterile fashion. The specimens will be divided into thirds. One third will be snap frozen in OCT medium and used for <u>in situ</u> hybridization (appendix 19.5) and immunohistochemistry (appendix 19.6). Another portion (1/3) will be snap frozen for extraction of messenger RNA for RT-PCR (appendix 19.7). The remaining third will be placed in tissue culture with 6000 IU/ml IL-2 and complete medium and expanded. Total lymphocytes and phenotype will be quantitated at 1 and 2 weeks. If cells expand well, cytotoxic assays and proliferation against autologous tumor will be evaluated. Serum will be tested for IL-12 levels and IFN-g levels.

4.7 Periodic Monitoring for Evidence of Replication Competent Retrovirus.

As recently required by the FDA, we will monitor this possibility periodically. We will perform serological assays for evidence of antibody to retroviral envelope proteins, assays for reverse transcriptase, and direct

assays for viral nucleic acid in peripheral blood leukocytes (PBL) using a PCR technique. We also mohnitor patients at least monthly during treatment, monthly for the first three months following completion of treatment, every three months for the remainder of the year following completion of treatment, and then at least yearly threafter. Inaddition, we will obtain and hold frozen sera and PBL from each patient at each time point in the monitoring program, and hold these samples indefinitely. For patients with positive results in any of the above assays, we will attempt to identify infectious retrovirus by direct culture of patient PBL. For this assay, we will perform a co-culture of the patient PBL with a cell line susceptible to retroviral infection such as NIH3T3 cells.

5.0 PATIENT SELECTION AND RECRUITMENT

5.1 Eligibility:

A total of 18 patients with cutaneous cancer lesion(s) will be enrolled in the preliminary study of the maximum tolerable or "possible" dosage study. Additional patients with these types of cancer (melanoma, breast cancer, and head and neck cancer) will be treated at the maximum toleraated dose only after the review of the initial experience and the approval by the IRB and the RAC. Fig. 6 shows the 95% confidence limits for response rate at the completion of treatment of 15, 25, or 35 patients.

A. Patients must have histologically confirmed metastatic, and/or unresectable, locally advanced melanoma, breast cancer, or head and neck cancer for which curative measures do not exist or are no longer effective. They should have accessible cutaneous tumors. Patients must have expected survivals of 12 months or less. Patients must be off all other antitumor therapy for at least 1 month prior to beginning this treatment.

B. Women of child-bearing potential must have a negative pregnancy test.

C. Patients must have a negative HIV test.

D. Patients must have intact cell mediated immunity as manifested by a positive response to any one of a broad panel of skin tests including TB, mumps, candida, and SKSD (Merieux skin test). Skin tests will be read at 48 hours.

E. Patients must be free of systemic infection.

F. Patients must have the following laboratory values:

1. WBC > $2000/mm^3$
2. Platelet count > 50,000
3. Bilirubin < 2mg/dl
4. Creatinine < 2mg/dl

G. Patients must have biopsy proven metastatic melanoma, breast cancer, or head and neck cancer that is measurable by physical exam, or radiologic immaging after biopsies of tumor material have been obtained.

H. Patients must be at least 18 years old.

5.2 Ineligibility:

A. Patients who fail to meet the above criteria.

B. Patients who are hepatitis B surface antigen positive or HIV antibody positive.

C. Patients who require steroids in any form- topical or systemic.

D. Patients who have had therapy within 28 days

E. Patients who are pregnant

F. Patients with systemic infections or coagulation disorders

G. Patients with an ECOG performance status >2.

5.3 Patient Recruitment

Patients will be recruited from those currently being evaluated at the University of Pittsburgh Medical Center. In addition information will be available through the Cancer Information Service (1-800-4-CANCER). All patients will have another physician not identifified with this protocol see them and participate in the decision of the patient to enter this experimental treatment. Patients will be given as much time as necessary to think about participation before signing the consent form but no less than one week.

6.0 LOCATION

All patients will be treated on the PCI inpatient or outpatient units of Montefiore University Hospital. All resected tissues, serum, and blood specimens will be processed by the IMDL/CT of the PCI located on the 10th floor of the BST at the University of Pittsburgh. Viral supernatant will be prepared in the HGTAL located in Biothechnology Center of University of Pittsburgh and approved for human use by the FDA.

7.0 PATIENT EVALUATION

7.1 Before Therapy:

A. Complete physical exam including detailed measurement of all metastatic lesions. Recording of the cutaneous lesion(s) to be injected will be performed including photography as necessary.

B. Blood tests (85 ml) including:

1. electrolytes, BUN, creatinine, AST, ALT, LDH, total bilirubin, direct bilirubin, alkaline phosphatase, glucose, calcium, magnesium, phosphorus, albumin, total protein, CEA.

2. CBC with differential and platelet counts.

3. PT, PTT.

4. Pregnancy test for women who may conceive.

5. Hepatitis screen

6. HIV screen

7. 10 ml of clotted blood for serum storage (IFN-g and IL-6 levels)

8. 45 ml of blood for mononuclear cell preservation.

C. Urinalysis

D. Chest X-ray

E. EKG

F. Baseline CT scan or MRI of brain, chest, abdomen, and pelvis to evaluate metastatic disease

G. Merieux skin test for DTH reactivity.

7.2 After Therapy:

A. Physical examination at 24 hours after the first injection and then weekly after each injection which will include an assessment of the injection sites.

B. Weekly blood tests (x 6) including:

1. CBC with differential and platelet counts.

2. Chemistries (see above)

3. PT, PTT

4. Serum (10ml) and PBMC (30ml) for storage

C. Biopsy of vaccination sites on day 28.

D. Merieux skin test for DTH reactivity at the time of biopsy.

E. Appropriate radiographic and physical examination on day 60 and 90 to assess tumor response.

8.0 CRITERIA FOR RESPONSE

Patients will be assessed at one and two months following their fourth vaccination for evidence of tumor response using physical examination or radiographic studies. Responses at uninjected sites will be defined as follows:

A. Complete response- disappearance of all measurable disease for at least 1 month without the development of new lesions.

B. Partial response- 50% or greater decrease in the sum of the products of the perpendicular diameters of all measurable lesions lasting at least 4 weeks with no increase in the size of existing lesions or appearance of new lesions.

C. No response- any patient not manifesting a complete or partial response.

9.0 REPORTING OF ADVERSE REACTIONS

All side effects per course will be graded using the standard toxicity sheet used in prior immunotherapy protocols presented in appendix 18.7. Adverse reactions will be reported promptly to the Investigational Drug Branch at (301) 496-7957. Reports are required even if there is only a suspicion of an adverse effect. Previously unknown grade 2 and grade 3 reactions will be reported to the NCI in writing using the "NCI Adverse Reactions Form for Investigational Agents" within 10 working days. Grade 4 (life-threatening) reactions and patient deaths while on treatment will be reported to NCI by phone within 24 hours. A written report will follow within 10 working days. Written reports will be sent to:

Investigational Drug Branch
Cancer Therapy Evaluation Program
P.O. Box 30012
Bethesda, Maryland 20824

Copies of adverse reactions reports will also be sent to the RAC. All adverse reactions should also be reported to the IRB. Data will be submitted to CTMS at least once every two weeks. The NCI/DCT case report or ACAS will be used to report to CTMS.

10.0 POTENTIAL RISKS OF IL-12 ADMINISTRATION AND RETROVIRAL-MEDIATED GENE MODIFICATION

10.1 Possible risks of IL-12

In murine studies, we have seen essentially no toxicity in doses of recombinant murine IL-12 when administered daily i.p. or s.q. for as long as seven days in C57BL/6 mice. Similar studies carried out by Brunda et al (1994) for up to 30 days with administration of IL-12 for five out of seven days again show effectively no major toxicity. Modest elevations in transaminase, reduction in serum protein and albumin and mild leucopenia and thrombocytopenia are observed. At the conclusion of IL-12 therapy there is no rebound phenomenon but rather a return to normal levels of formed elements. Extramedullary hematopoiesis and anemia (approximately 20% reduction in Hct at Decreased appetite and body weight as well as mortality were observed only at doses > or = to 500 mg/kg/day.

In primate studies, mortality in a single cynomologus monkey was observed after seven days of treatment with 50 mg/kg/day (T. Anderson, Hoffmann-La Roche). In squirrel monkeys, doses up to 50 mg/kg/day were well tolerated. Some evidence of weight gain attributable to a vascular leak phenomenon, and formation of antibodies to hrIL-12 in these animals were observed. Toxicities in the other cynomologus monkeys revealed only very mild toxicity at the 1 mg/kg/d x 14 days dose with minimal loss of appetite and diarrhea at the 10 mg/kg/day dose.Mild (less than two fold) eleveations in hepatic transaminase was noted at the 1 mg/kg/day level. Preliminary findings in cynamologus monkeys (Data on file, Genetics Institute) showed that following the single IV bolus, serum concentrations of IL-12 exhibited a multiphasic decline with an apparent terminal half-life ranging from

approximately 15.1-27.6 hours in males and from 9.9-15.5 hr. in females. The estimated systemic clearance (CLT) averaged 6.7 and 8.0 ml.hr.kg. for males and females, respectively. Estimated from the limited data, the apparent half-life following the SC dose was longer than that observed for the IV route, ranging from 26.6 hrs -48.3 hrs. in males and 9.7-19.7 hrs in females, presumed due to continuous absorption. Compared to the IV data for each animal, the estimated absolute bioavailability (F) of the SC dose ranged from 14-29% in the 6 nonhuman primates studied (data on filw, Genetics Institute and Hoffmann-La Roche; Minutes of Advisory Board Meeting for IL-12, New York; February 9, 1993). We would presume half life kinetics obtained with fibroblast delivery comparable to that observed with subcutaneous administration.

10.2 Insertional Mutagenesis:

The possibility of causing malignancy in cells secondary to the random insertion of the retroviral vectors in the genome exists, though the actual risk of this occurring is thought to be low. Nevertheless, transduced fibroblasts will be irradiated with 5,000 rads prior to vaccination. Tests of the viral supernatant as well as of the fibroblasts used for vaccination will be conducted to assure that no replication competent virus is present in either preparation. Since no other cells will be exposed to retroviral vector insertion, no other cells will be at risk for insertional mutagenesis.

10.3 Risk from Murine Retrovirus:

Exposure of the cancer patient to retrovirus could theoretically pose a risk of insertional mutagenesis. It should be emphasized, however, that careful tests will be conducted to assure that the patient is not exposed to replication competent virus. The retrovirus derived from the Moloney murine leukemia virus has been modified so that it no longer contains any intact viral genes and thus cannot produce the proteins necessary to package its RNA into an intact infectious virus (14, 15). To assemble the retrovirus, a second-generation retrovirus packaging cell line (YCRIP) will be used that contains coding sequences that express the viral structural proteins in two separate plasmids. This packaging cell line does not produce replication competent retrovirus because of multiple modifications made to the gag, pol, and env coding sequences that prevent its replication. These modifications include removal of signals required for RNA encapsidation, priming of reverse transcription, and integration (Y region). Multiple assays will be performed on the packaging cell line, the retroviral vector supernatant as well as on the fibroblasts prior to vaccination to insure that no replication competent virus is present. These tests will include S+/L- assays and 3T3 amplification. Any supernatants or fibroblasts with evidence of any replication competent virus will not be utilized. The 3T3 amplification and S+/L- assays are thought to be capable of detecting a single replication competent viral particle per ml. These studies will initially be done at HGTAL and then subsequently in the IMDL/CT.

Prior safety studies have shown that exposure of primates to large infusions of infectious murine amphotrophic virus produce no acute pathologic effects (16). In a study of 21 primates receiving retroviral-mediated, gene-modified, autologous bone marrow cells no animal showed evidence of toxicity related to the gene transfer for as long as 4 years after infusion (17).

Patients in the proposed protocol will not be exposed to the vector

supernatant. Fibroblasts will be transduced with the retroviral vector supernatant and then washed extensively. They will be grown for several weeks in the absence of supernatant. The fibroblasts will then be washed extensively again prior to reinfusion into the patient. Taking into consideration that the half life of the retroviral vector in vitro is 6 to 8 hours at 37 ^{o}C, and the extensive washing of cells, the propagation is unlikely to be contaminated with infectious retroviral particle in cells for injection.

11.0 STATISTICAL CONSIDERATIONS

11.1 Analysis of Peripheral Blood Specimens:

Systemic IL-12, IFN-g, and IL-6 levels and the phenotype of circulating peripheral blood lymphocytes will be determined on days 0 (pre-vaccination), 7, 14, 21, 28, 35 and 42. Follow-up multiple comparisons, based on either the paired-t or signed rank statistic, will be applied in the interpretation of significant results.

11.2 Analysis of Local Toxicity:

The incidence of severe, local reactions will be recorded. The relationship between local toxicity and the number of transferred cells and the amount of IL-12 elaborated per site per day will be determined.

11.3 Clinical Response:

The limited number of patients to be accrued (18) during the first phase of evaluation together with their varied histologies (3) will limit the generalizability of the response rates that will be observed. Rates of response will be tabulated and compared to relevant norms. In the second phase of the evaluation, a total of 15 patients with each of these separate histologic tumor types (breast cancer, T-cell lymphoma, melanoma, and head and neck cancer) will be evaluated.

12.0 ETHICAL CONSIDERATIONS

Dr. Rosa Lynn Pinkus (Medical Ethics) will assist in developing and reviewing all protocol consent documents with the principal investigators. Every attempt will be made to protect the patients' and their family's privacy. Informed consent in this project is considered to be a process of shared decision-making between patient and physician. All written consents will be discussed with this concept in mind.

13.0 RESPONSIBILITIES

13.1 Consent- Drs. Tahara or Lotze.

13.2 Harvest of the skin- Drs. Tahara or Lotze.

13.3 Peritumoral injection of fibroblasts - Drs. Tahara or Lotze.

13.4 Biopsy of vaccination sites- Drs. Tahara, Lotze, Rubin, Edington, or Salup or fellows under supervision.

13.5 Preparation and culturing of fibroblasts, retroviral gene transduction- Immunologic Monitoring and Cellular Therapy Laboratories assisted by Dr. Tahara and Zitovogel.

13.6 Preparation of viral supernatants and safety testing- HGTAL/PCI assisted by Drs. Tahara and Zitovogel.

14.0 RISKS AND BENEFITS

Adverse effect will be recorded and available in the clinical protocol of systemic interleukin-12 which preced this gene therapy protocol. However, it is unlikely that severe and life threatening toxicities will be induced by the low levels of IL-12 elaborated locally from the injection sites.

Individual patients may benefit by having their tumors shrink or disappear. Symptoms related to cancer may improve if shrinkage of tumor is achieved. The population of patients with cancer may benefit from this trial which may define the role of *in vivo* tumor vaccine using cells genetically modified to produce cytokines such as IL-12.

If there are serious systemic effects of the IL-12 gene therapy, which we believe are unlikely to occur, the injection site can be surgically excised. As an additional safety factor, fibroblasts have been shown to stop producing transfected gene products in murine models after one to two weeks.

15.0 COST AND PAYMENTS

Patients will be expected to provide insurance coverage for payments for all routine and subsequent care, and for any sickness or problems that may arise out of the nature of your disease. They will be charged as for routine treatments for all outpatient visits, hospitalization, routine blood samples and other tests performed to evaluate the course of tumor prior to and following this program of therapy. Gene transfection procedure and biopsies necessary for the experimental portion of this protocol will be supplied free of charge. This institution will bear the acute costs of non-negligent injuries arising out of the experiment. Cost will not be a factor in determining who will be treated. The cost of the experimental therapy will be born by the University of Pittsburgh.

16.0 TREATMENT SCHEMA

DAY	0	1	2	3	4	5	6	7	8/	/14/	/21/	/28/	/35	42
INJECTION	X							X		X	X			
BIOPSY												X		
BLOOD	X							X		X	X	X	X	X
CXR	X							X		X	X	X	X	X
PHYS EXAM	X	X						X		X	X	X	X	X

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CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

TITLE: IL-12 Gene Therapy Using Direct Injection of Tumors with Genetically Engineered Autologous Fibroblasts (94-21).

PRINCIPAL INVESTIGATORS: Hideaki Tahara, M.D., Ph.D.
Research Assistant Professor of Surgery
University of Pittsburgh
School of Medicine
Pittsburgh, PA 15261
(412) 648-9489

Michael T. Lotze, M.D.
Director of Surgical Oncology
Pittsburgh Cancer Institute
Professor of Surgery
University of Pittsburgh
School of Medicine
Pittsburgh, PA 15261
(412) 648-9489

CO-INVESTIGATORS: Paul D. Robbins, Ph.D.
Assistant Professor
Molecular Genetics and Biochemistry
University of Pittsburgh
School of Medicine
(412) 648-9268

Walter J. Storkus, Ph.D.
Assistant Professor of Surgery
Molecular Genetics and Biochemistry
University of Pittsburgh
School of Medicine
(412) 624-9375

Laurence Zitvogel, M.D.
Research Fellow
Molecular Genetics and Biochemistry
University of Pittsburgh
School of Medicine
(412) 624-9375

Howard Edington, M.D.
Assistant Professor of Surgery
668 Scaife Hall
(412) 648-9489

Jeffrey Myers, M.D., Ph.D.
Research Fellow

Patient's Initials

Surgical Oncology
Pittsburgh Cancer Institute
University of Pittsburgh
School of Medicine
(412) 624-9375

Chet Nastala, M.D.
Research Fellow
Surgical Oncology
Pittsburgh Cancer Institute
University of Pittsburgh
School of Medicine
(412) 624-9375

Itzhak Pappo, M.D.
Research Fellow
Surgical Oncology
Pittsburgh Cancer Institute
University of Pittsburgh
School of Medicine
(412) 624-9375

Joshua Rubin, M.D.
Assistant Professor of Surgery
University of Pittsburgh
School of Medicine
Pittsburgh, PA 15261
(412) 648-9489

Herbert J. Zeh, III, M.D.
Intern
Surgical Oncology
Pittsburgh Cancer Institute
University of Pittsburgh
School of Medicine
(412) 624-9375

John M. Kirkwood, M.D.
Associate Director for Medical Oncology
Chief, Division of Medical Oncology
Pittsburgh Cancer Institute
Pittsburgh, PA 15213
(412) 648-6570

Theodore Logan, M.D.
Pittsburgh Cancer Institute
Division of Medical Oncology
Montefiore University Hospital
3459 Fifth Avenue, Main North
Pittsburgh, PA 15213
(412)648-6575

Patient's Initials

Raoul Salup, M.D.
Assistant Chief of Urology
VA Hospital
University Drive C
Pittsburgh, PA 15240
(412) 692-3552

Maury Rosenstein, M.D.
Department of Radiation Oncology
University of Pittsburgh Medical Center
(412) 647-3600

SOURCE OF SUPPORT: **None**

INTRODUCTION AND BRIEF DESCRIPTION: I have been invited to participate in a study to see if I can benefit from being injected with a preparation made from my own skin. I am aware that my tumor has spread beyond the limits that are curable by surgery or other standard treatments. While it is not possible to predict whether I will receive any personal benefit from my participation in this study, information may be obtained that will be helpful to others with similar disease. To be eligible for this protocol, I certify that I am not pregnant or planing to get pregnant. In addition , I will use barrier forms of contraception during the treatment.

Before beginning treatment, my physician will perform a history and physical examination. Routine physical examination will include evaluation of the heart, lung, abdomen, head and neck and extremities. This will also include measurement of all skin lesions. I will have blood tests (approximately 4 tablespoons of blood will be drawn), a urine test, a chest x-ray, an electrocardiogram to check my heart, and CT or MRI scans (special x-rays) performed. Blood (4 tablespoons) will be drawn on days 0 (pre-treatment), 7, 14, 21, 28, 35 and 42. These blood tests are to look at cells in my blood called lymphocytes and the level of IL-12 in my blood. Skin tests will be done to determine how my immune system is working. The Merieux (Pasteur-Merieux) skin test is commercially available from the pharmacy and is routinely used on patients to determine if their immune system reacts to the skin test. I will also be given a small injection of my tumor cells that have been irradiated to determine if my immune system reacts to my tumor cells.

I understand that in order to perform this study, fibroblasts will be prepared. These are cells that are normally found in the skin. In order to obtain these cells, a small piece of my skin (1/2 inch wide and 2 inches long) will be removed. Little discomfort will be associated with the skin removal. The area will be injected with a medication to numb the area and then the skin will be surgically removed. A few stitches may be used to close the area. A clean dressing will be applied. I will be monitored closely and treated for any problems that may occur. This will be done at the time when my doctors are getting tissue samples for the diagnosis of my tumor. When enough of these cells have been obtained, my doctors will take a mouse virus, which contains a human gene (interleukin-12), and then insert these into the fibroblast cells from the skin. This virus has been developed in the laboratory to contain the human gene which can alter immune reaction of the human body. The mouse virus appears to be applicable to human, this is based on current information that could change in the future. This will be used as a "transportation system", and will also contain a substance which can be recognized by special testing. This new gene will enable my fibroblast skin cells to make a normal human protein called interleukin-12 (IL-12). IL-12 is usually made in very small amounts only by cells of my immune system. Interleukin-12 is thought to stimulate an immune response to the tumor cells in the preparation. This immune response may make my tumors shrink.

Approximately 3-4 weeks after the skin biopsy has been done, the fibroblasts that are making interleukin-12 will be injected into one or two of my tumors that are easily accessible, such as those lying in the skin using a very small needle. These sites will be tattooed (very tiny marking) with a substance called india ink so that their location will be known. Each site will contain fibroblasts that are either making or not making interleukin-12. I understand that I must return to the Pittsburgh Cancer Institute Outpatient Services the next day after these injections for the purpose of seeing if I am having any side effects.

_____
Patient's Initials

One week after the first injection, I will have the same procedure done except that I will not be injected with the fibroblasts NOT secreting interleukin-12. This injection will be repeated two weeks after the first injection and three weeks after the first injection.

Twenty eight days after the first injections, two injection sites will be biopsied (a small piece of tissue removed) under local anesthesia. These biopsies will each require a few skin stitches. The pieces of skin that are removed will be examined to see if this treatment is working. I will also have a physical examination at this time.

Lastly, I understand that I must return to the Pittsburgh Cancer Institute Outpatient Services one and two months after the last injections to evaluate my progress.

A total of 18 patients with cutaneous (skin) cancer lesions will be enrolled in the first part of this study which will be looking to see the highest dose humans can tolerate. All patients will be treated on the Pittsburgh Cancer Institute Inpatient or Outpatient Units.

RISKS AND BENEFITS: The potential benefit of this treatment lies in the possibility that my tumor will shrink or disappear. If this happens, I may or may not live longer. Symptoms caused by my tumors may get better after therapy. It is not possible to predict whether I will receive any personal benefit from my participation in this study.

There is a small risk associated with skin biopsy that includes infection, bleeding, and delayed healing. Both of these complications are easily treated. The risks of injections with my own skin cells may include soreness and/or bleeding at the injection site. Local inflammation and/or discomfort due to lymph node enlargement may occur. On occasion, persons reacting strongly to the preparation might develop local ulcers or infection.

Interleukin-12 could cause an elevation in transaminase, an enzyme secreted by my liver, decreased white blood cells, which may increase my risk of infection; decreased platelets, which may cause problems with blood clotting, increasing my risk of bruising or bleeding; increased protein and albumin, which may cause swelling, extra-medullary hematopoiesis, which means my blood cells may form outside my bone marrow. Anemia, weight loss, decreased appetite are rare, but may occur.

There is a slight risk that this treatment may cause a second cancer in my body, although the chance of this is low. The fibroblasts will be pretreated with x-rays to prevent their growth after being injected into my skin. The mouse virus used to put a new gene into my fibroblasts has been treated in such a way that it is nearly impossible that it could infect other cells in my body or cause a new tumor to grow.

In the event of death, wheather from accident, injury or illness, I have been informed that an autopsy will be requested. My close relatives have been informed of my wishes.

ALTERNATIVE TREATMENTS: Alternatives that could be considered in my treatment include experimental chemotherapeutic drugs alone or in combination. The principal investigators of this study have provided me with detailed information about my disease, and the benefits and risks of other available treatments. I should feel free to discuss my disease and my prognosis with the investigators. The physician involved in my care will be available to answer any questions I may have regarding this program.

NEW INFORMATION: If any information, either good or bad, comes to the attention of the investigators which may relate to my willingness to continue to participate, it will be provided to me. I have been told that should my disease become worse, should new scientific developments occur that indicate this treatment is not in the best interest, or should my physician feel that this treatment is no longer in my best interest, the treatment will be stopped. Further treatment would be discussed.

COSTS AND PAYMENTS: The gene transfection procedure and biopsies necessary for this study will be supplied free of charge. This institution will bear the acute costs of non-negligent injuries arising out of the experiment. Cost will not be a factor in determining who will be treated. I, or my insurance company, will be responsible for payments for all routine and subsequent care, any sickness or problems that may arise out of the nature of my

disease, outpatient visits, hospitalization, routine blood samples and other tests to evaluate the course of my tumor prior to and following therapy. The cost of the experimental therapy will be born by the University of Pittsburgh.

CONFIDENTIALITY: A record of my progress while on the study will be kept in a confidential form in the Pittsburgh Cancer Institute. During reviews of this data, which are required by the Food and Drug Administration as well as the National Cancer Institute, access will be provided to my medical records that might reveal my identity. In addition, the sponsor of this protocol will have access to my medical records that contain information regarding my identity. My medical records may be reviewed by qualified members of the Pittsburgh Cancer Institute. Copies of information contained in my record, such as laboratory test results, will be made for my research record. However, no information by which I can be identified will be released or published.

My records, like hospital records, may be subpoenaed by court order. I consent to publication of the results of this study, so long as I am not identifiable by name in such publications.

RIGHT TO WITHDRAW: Participation in this study is voluntary. I am free to withdraw my consent to participate in this treatment program at any time without prejudice to my subsequent care. The doctors have urged me not to withdraw so that I can be safely observed, especially during the period immediately after the immunizations. I realize that I should receive careful medical follow-up of the immunization sites. If I withdraw from the study I should have other physicians involved in my care notified of this treatment. Refusing to participate will involve no penalty or loss of benefits. I am free to seek care from a physician of my choice at any time. Life-long follow up will be required and I will inform the investigators of my current address and phone number in the event that I move.

COMPENSATION FOR ILLNESS OR INJURY: I understand that in the event of physical injury or illness resulting from this research procedure no monetary compensation will be made or provision for long term medical care. Any immediate emergency medical treatment including hospitalization that may be necessary will be provided.

VOLUNTARY CONSENT: I certify that I have read the proceeding or that it has been read to me, and I understand its contents. Any questions I have pertaining to the research have been, and will continue to be answered by the investigators listed on the front sheet of the consent form. Any questions I have concerning my rights as a research subject will be answered by the Office of the Senior Vice Chancellor, Health Sciences. A copy of this consent form will be given to me. My signature below means that I freely agree to participate in this experimental study.

_____ _____

Date Patient's Signature

I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participating in this research study, have answered any questions that have been raised, and have witnessed the above signature.

_____ _____

Date Investigator's Signature

_____

Witness's Signature

3/28/94

_____

Patient's Initials

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Michael T. Lotze, M.D.	Professor of Surgery, Molecular Genetics and Biochemistry, Associate Director, Pittsburgh Cancer Institute/Surgical Oncology

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
N.U. Honors Program in Medical Education, Evanston, IL	B. Med. Sci.	1973	Premedical Studies
Northwestern Medical School, Chicago, IL	M.D.	1974	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES. GT/93

Positions

1975	Jr. Medical Fellow in Surgery, M.D. Anderson Tumor Institute, Houston, TX
1975-1982	Intern/Resident in Surgery, Strong Memorial Hospital, Rochester, NY
1977-1978	Medical Officer, National Health Service Corps, Onamia, MN
1978-1980	Staff Fellow, Surgery Branch, National Cancer Institute, Bethesda, MD
1982-1990	Senior Investigator, Surgery Branch, National Cancer Institute, Bethesda, MD
1990-Pres.	Director, Division of Surgical Oncology, Department of Surgery and Associate Director, Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA

Awards and Honors:

1971-1974	Honors Program in Medical Education, Northwestern University
1982	Robert H. White Award for Excellence in Teaching, University of Rochester
1986	4th Annual Edith Hamilton Cancer Lecturer, Rochester, New York
1987	Special Achievement Award; DHHS, NIH
1988	4th Vender Lecturer, Northwestern University, Evanston, Illinois
1990, 1993	Coorg. Keystone Symposium on Cellular Immunity and the Immunotherapy of Cancer, Park City, UT, Taos NM
1990	Virginia Mason Research Center Distinquished Lecturer, Seattle, WA
1991	Annual John Palmer Lecturer, University of Toronto; Toronto, Ontario
1991	Tenth Hinshaw Lecturer, University of Rochester, Rochester, NY
1992	Coorganizer Keystone Symposium on Melanoma and Biology of the Neural Crest, Taos, NM

Activities

1988-1990	Surgery Br. Rep., NCI Investigational Review Board
1990	Ad Hoc Reviewer, Experimental Immunology Study Section, NIH
1990	Chairman, Special Study Section, Experimental Immunology, NIH
1991	SSO/Program Committee/Research and Government Relations Committee
1992-1995	ASCO/Young Investigator Award, Clinical Development Award Committee

Editorial Boards:

1988 - 1992	European Cytokine Journal; Journal of Immunotherapy (Assoc. Editor); Journal of Immunology; Contemporary Oncology; Melanoma Research

Publications (out of 195)

1. Lotze, M.T., Strausser, J.L., Rosenberg, S.A.: In vitro growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. J. Immunol. 124:2972-2978, 1980.
2. Kawakami, Y., Custer, M.C., Rosenberg, S.A., Lotze, M.T.: Interleukin-4 regulates Interleukin-2 induction of lymphokine activated killer activity from human lymphocytes. J. Immunology. 142:3452-3461, 1989.
3. Kawakami, Y., Rosenberg, S.A., Lotze, M.T.: IL-4 promotes the growth of tumor infiltrating lymphocytes specific for human autologous melanoma. J. Exp. Med., 168:2183-2191, 1988.
4. Custer, M.C., Lotze, M.T.: A biologic assay specific for IL-4: Rapid Fluorescence Assay for IL-4 detection in supernatants and serum, J. Imm. Methods, 128: 109-17, 1990.
5. Kasid, A., Morecki, S., Lotze, M.T., et al.: Human Gene Transfer: Characterization of human tumor infiltrating lymphocytes as vehicles for retroviral mediated gene transfer in man. Pro. Nat. Acad. Sci., 87:473-477, 1990.

Kawakami, Y., Haas, G.P., Lotze, M.T.: Expansion of Tumor Infiltrating Lymphocytes from Human Tumors Using the T cell Growth Factors IL-2 and IL-4. J. Immunotherapy, in press.

7. Stötter, H., Custer, M.C., Lotze, M.T., et al.: IL-7 induces human lymphokine activated killer (LAK) cells and is regulated by interleukin-4. J. Immunol 146: 150-144, 1991.
8. Rosenberg, S.A., Aebersold, P., Lotze, M.T., et al.: Gene transfer into humans: Immunotherapy of patients with advanced melanoma using tumor infiltrating lymphocytes modified by retroviral gene transduction. N. Engl. J. Med. 323: 570-578, 1990.
9. Stötter, H. and Lotze, M.T.: Human Lymphokine Activated Killer Activity: Role of IL-2, IL-4, and IL-7. Arch Surg 126:1525-30, 1991.
10. Wong, H. L., Lotze, M.T., Wahl, L.M., et al.: Administration of recombinant IL-4 to humans regulates gene expression, phenotype, and function in circulating monocytes. J. Immunol. 148:2118-2125, 1992.
11. Rubin J. T. and Lotze M.T.: Acute Gastric Mucosal Injury Associated with Systemic Administration of IL-4. Surgery 111:274-280, 1991.
12. Lotze MT. Role of IL-4 in the antitumor response in H. Spits,ed. IL-4 Raven Press, 1992, Chapter 15, 237-262.
13. Lotze MT. Trials and Tribulations: The Cost of Developing Gene Therapies FASEB Jl 5:3013-3014, 1991.
14. Lotze MT. Clinical Protocol: The treatment of patients with melanoma using IL-2, IL-4, and tumor infiltrating lymphocytes. Human Gene Therapy, 3: 167-177, 1992.
15. Lotze MT, Zeh HJ, Elder EM, et al.: Use of T-cell Growth Factors (IL-2 to IL-12) in the investigation and treatment of melanoma. J. Immunotherapy, 12: 212-217, 1992
16. Weidmann E, Elder EM, Lotze, MT., et al.: Usage of the T-Cell receptor Vβ chain genes in fresh and cultured tumor-infiltrating lymphocytes from human melanoma. Int. J. Cancer 54:383-390, 1993.
17. Tahara H, Mueller GM, Ricordi C, Robbins PD, Lotze MT. Islet cell transplantation facilitated by gene transfer. Transplant. Proc. 24:2975-2976, 1992.
18. Wong HL, Costa GL, Lotze MT., et al.: IL-4 differentially regulates monocyte IL-1 family gene expression and synthesis in vitro and in vivo. J Exp Med, 177:775-781, 1993.

Sanda MG, Yang JC, Lotze MT., et al.: Intravenous administration of recombinant human macrophage colony-stimulating factor to patients with metastatic cancer: A Phase I Study. J Clin Onc, 10: 1643-1649, 1992.

20. Robbins PD, Tahara H, Lotze MT., et al.: Retroviral Vectors for use in Human Gene Therapy for Cancer, Gaucher Disease, and Arthritis. Annals of the New York Acad. Sci., in press.
21. Zeh HJ, Hurd S, Lotze MT., et al.: IL-12 Promotes the Proliferation and Cytolytic Maturation of Immune Effectors: Implications for the Immunotherapy of Cancer. J Immunotherapy, in press, 1993.
22. Zeh HJ, Tahara H, and Lotze MT. Interleukin-12. Cytokine Handbook, Editor, A. Thomson, 1994.
23. Nastala C, Edington HD, Lotze MT., et al.: Recombinant IL-12 (r-mIL-12) Mediates Regression of Both Subcutaneous and Metastatic Murine Tumors. Surgical Forum. 44:518-521, 1993.
24. Edington H, McKinney TG, Lotze MT., et al.: Recombinant IL-12 Induces Tumor Regression in Murine Models: Interferon-Gamma But Not Nitric Oxide Dependent Effects. Submitted, 1993.
25. Tahara H, Pappo I, Lotze MT., et al.: Fibroblasts Genetically Engineered to Secrete IL-12 can Suppress Tumor Growth In Vivo and Induce Antitumor Immunity To A Murine Melanoma. Submitted
26. Lotze MT. Transplantation and Adoptive Cellular Therapy of Cancer: The Role of T cell Growth Factors. Cell Transplantation, 2:33-47, 1993.
27. Storkus W. J., Salter R. D., Lotze, M.T., et al.: Identification of T cell epitopes: Rapid isolation of class I-presented peptides from viable cells by mild acid elution. J. Immunother., in press, 1993.
28. Storkus W. J., Zeh III, H. J., Lotze, M.T., et al.: Isolation of human melanoma peptides recognized by class I restricted tumor infiltrating T lymphocytes. J. Immunol., in press, 1993.
29. Marincola FM, Rubin JT, Lotze MT., et al.: HLA association with response and toxicity in melanoma patients treated with iL-2 based immunotherapy. Cancer Research 52:6561-6, 1992.
30. White MV, Igarashi Y, Lotze MT., et al.: Effects of invivo administration of IL-2 and IL-4 alone and in combination, on ex vivo human basophil histamine release. Blood 79:1491-5, 1992.
31. Lotze MT, Finn OJ. Cellular immunity and the immunotherapy of cancer. J. Immunotherapy, 14:79-87, 1993.
32. Hunt, JD, Landreneau RJ, Lotze MT., et al.: Transfer and expression of the human IL-4 gene in carcinoma and stromal cell strains derived from lung cancer patients. J Immunotherapy, in press.

Lotze MT, Yang JC, Topalian SL, et al.: Prospective randomized trial of high-dose IL-2 alone or in conjunction with LAK cells for the treatment of patients with advanced cancer. Jl. Nat. Cancer Inst. 85:622-32, 1993.

federal register

Tuesday
July 5, 1994

Part III

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of actions under the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).

SUMMARY: This notice sets forth four actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: Today four actions are being promulgated under the NIH Guidelines. These four proposed actions were published for comment in the **Federal Register** announcements of August 11, 1987 (52 FR 29800); April 18, 1988 (53 FR 12752); December 30, 1988 (53 FR 53262); April 29, 1991 (56 FR 19776); November 9, 1993 (58 FR 59612); and February 11, 1994 (59 FR 6702). These proposed actions were reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meetings on September 21, 1987; December 3, 1988; January 30, 1989; May 30–31, 1991; December 2–3, 1993; and March 3–4, 1994.

In accordance with Section IV-C-1-b of the NIH Guidelines, these actions have been found to comply with the NIH Guidelines and to present no significant risk to health or the environment.

A revised version of the NIH Guidelines is published in a separate section of the **Federal Register** following this announcement. These revised NIH Guidelines differ from the previous version promulgated on May 7, 1986 (51 FR 16958) by incorporating within them the major actions to the NIH Guidelines that were promulgated on August 24, 1987 (52 FR 31848); July 29, 1988 (53 FR 28819); October 26, 1988 (53 FR 43410); March 13, 1989 (54 FR 10508); March 1, 1990 (55 FR 7438); September 12, 1990 (55 FR 37565); July 18, 1991 (56 FR 33174); October 15, 1991 (56 FR 51784); November 21, 1991 (56 FR 58800); January 28, 1992 (57 FR 3212); April 22, 1992 (57 FR 14774); August 26, 1992 (57 FR 38734); February 18, 1993 (58 FR 9102); April 23, 1993 (58 FR 21738); September 13, 1993 (58 FR 47906); October 18, 1993 (58 FR 53814); and the changes that are promulgated in this announcement.

I. Background Information and Decision on Action Under the NIH Guidelines

A. Amendment to Sections II, III–C, III–D, V, Appendices C–I, and G and Addition of Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants, and Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals of the NIH Guidelines

The NIH Guidelines were originally developed to cover research in laboratories in which recombinant DNA techniques were used. It is recognized today that these techniques are being used by scientists working with plants and large animals, and that procedures for containment of these plants and animals have not been specifically described in the NIH Guidelines. Institutional Biosafety Committees (IBCs) have requested guidance on the containment procedures that should be recommended for specific experiments with these organisms since they have the responsibility of approving such experiments under containment appropriate for the organisms. The principles of biological safety that are used to categorize experiments involving microorganisms, for example, are equally applicable to plants and animals. These safety procedures have been employed successfully for many years and have been recognized for their efficacy in biological containment.

Appendices P and Q are the result of several years of meetings and discussions involving research scientists and representatives from university, government, and industrial research sectors with expertise in several disciplines, including plant genetics, plant physiology, plant pathology, entomology, animal (including arthropod and aquatic species) physiology and reproduction, molecular biology, veterinary medicine, and human biomedical research. The Federal agencies involved in the development of Appendices P and Q include the NIH, the National Science Foundation (NSF), and the U.S. Department of Agriculture (USDA).

The process of developing Appendices P and Q was initiated when the USDA published an Advanced Notice of Proposed USDA Guidelines (USDA Guidelines) in the **Federal Register** on June 26, 1986 (51 FR 23367). This notice was followed by an announcement by the USDA regarding its intent to propose new guidelines for conducting all phases of research with domestic agriculture species, including both plants and animals modified through the application of genetic engineering techniques, in the **Federal Register** on December 9, 1986 (51 FR 44397). At that time, the NIH Guidelines did not include specific descriptions for containment conditions for research involving recombinant DNA containing whole plants and animals. The USDA convened a working group composed of university, government, and industrial scientists on December 13–14, 1986, with the purpose of discussing and redrafting guidelines for physical and biological containment of transgenic plant and animal species, and associated microorganisms. This meeting came to be known as the "Arlington House Workshop."

Participants of the "Arlington House Workshop," including former members of the RAC, agreed that the USDA Guidelines should be incorporated into the NIH Guidelines. The workshop participants noted that merging these two documents would offer the distinct advantage of providing a single comprehensive source of information regarding conduct of research involving organisms containing recombinant DNA and plants and animals exposed to microorganisms containing recombinant DNA.

A staff working group representing the Office of Recombinant DNA Activities, NIH, and the Cooperative State Research Service, USDA, held meetings during the following six months. This working group met with the purpose of revising the containment section and developing a final incorporated document for RAC review, approval by the NIH Director, and incorporation into the NIH Guidelines.

On June 28, 1987, and July 16, 1987, the RAC appointed the Working Group on Revision of the NIH Guidelines to meet and consider the draft documents, Appendices P and Q, and minor modifications to the NIH Guidelines, that would accommodate the proposed appendices. Appendices P and Q and the proposed revisions to the NIH Guidelines were published for public comment in the **Federal Register** on August 11, 1987 (52 FR 29800). Additional revisions to Appendices P and Q were proposed by the RAC and the Agricultural Research Service, USDA, at the September 17, 1987, RAC meeting. These modifications were

published for public comment in the Federal Register on December 30, 1988 (53 FR 53262). The RAC Working Group on Transgenic Animals proposed additional modifications to Appendices P and Q which were published for public comment in the Federal Register on April 18, 1988 (53 FR 12752). Further revisions were approved by the RAC at its January 30, 1989, meeting.

Throughout all of the meetings, discussions, and revisions, the intent of the Federal agencies and interested parties has been to describe working conditions that would minimize the risk to both the researcher and the environment from any possible harm or adverse effects due to the conduct of research involving recombinant DNA containing organisms.

On June 24, 1994, an Environmental Assessment of Appendices P and Q was completed by the NIH and USDA, and there was a finding of no significant impact. Copies of the Environmental Assessment are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

B. Amendment to Sections I-C-1-b-(2) and Deletion of Section III–A–2 of the NIH Guidelines Regarding Deliberate Release

On December 6, 1990, the RAC Planning Subcommittee recommended that the requirement for RAC review of experiments involving deliberate environmental release of organisms containing recombinant DNA be eliminated from the NIH Guidelines. This recommendation reflects the fact that the Federal regulatory agencies, the USDA, and the Environmental Protection Agency (EPA), are responsible for the review and approval of environmental release experiments. The proposed amendment was published for public comment in the Federal Register on April 29, 1991 (56 FR 19776). The RAC reviewed and recommended approval of the proposed amendment at its May 30–31, 1991, meeting.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

C. Amendments to Sections I, III, IV, and V, and Appendix M of the NIH Guidelines Regarding NIH/ORDA Review and Approval of Certain Categories of Human Gene Transfer Experiments That Qualify for the Accelerated Review Process

On December 3, 1993, and March 3–4, 1994, the Working Group on Accelerated Review Protocols presented an overview of the proposed amendments to the NIH Guidelines. The proposed amendments will: (1) Establish an accelerated review process for certain categories of human gene transfer experiments, (2) allow the NIH/Office of Recombinant DNA Activities to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with the NIH Guidelines, (3) allow the NIH/Office of Recombinant DNA Activities to approve those categories of human gene transfer experiments that qualify for the accelerated review process in consultation with the Chair and one or more RAC members, as necessary, and (4) exempt certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects which are not covered by Section V–U. All human gene transfer experiments approved by the NIH/Office of Recombinant DNA Activities through the accelerated review process will be provided in a report by the RAC Chair at the next regularly scheduled RAC meeting and will be included in the list of approved experiments which is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

The proposed amendments were published for public comment in the Federal Register on November 9, 1993 (58 FR 59612) and February 11, 1994 (59 FR 6702). The RAC reviewed and unanimously recommended approval of the proposed amendments at its March 3–4, 1994, meeting.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

D. Amendments to Section V–U of the NIH Guidelines Regarding Recombinant DNA Vaccines

On March 3, 1994, the Working Group on Vaccines presented an overview of the proposed amendment to the footnote in Section V–U. The proposed amendment will define those categories of experiments involving the administration of recombinant DNA vaccines that are exempt from RAC review and NIH and Institutional Biosafety Committee approval.

The proposed amendment was published for public comments in the Federal Register on February 11, 1994 (59 FR 6702). The proposed amendment was revised by the RAC at its March 3–4, 1994, meeting. The revised amendment was unanimously approved.

The action is detailed in Section II—Summary of Actions. I accept this recommendation, and the NIH Guidelines will be amended accordingly.

II. Summary of Actions

A. Amendment to Section I, Scope of the NIH Guidelines

The amended version of Section I reads as follows:

Section I. Scope of the NIH Guidelines

Section I–A. Purpose

The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) Recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I–A–1. Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, has been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval (see exceptions in Sections I–A–2 and I–A–3).

Section I–A–2. Certain experiments that involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) shall be considered Major Actions (see Section IV-C-1-b-(1)), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review (see Section III–A–2).

Section I–A–3. Experiments involving the transfer of recombinant DNA to one or more human subjects that are not considered under Section III–A–2 may qualify for Accelerated Review (see Section III–B–2 and Appendix M–V) and will be considered as Minor Actions (see Section IV-C-1-b-(2)-(a)). Actions that qualify for Accelerated Review will be reviewed and approved by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary.

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III–C–7).

Section I–B. Definition of Recombinant DNA Molecules

In the context of the NIH Guidelines, recombinant DNA molecules are defined as either: (i) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

Section I–C. General Applicability

Section I–C–1. The NIH Guidelines are applicable to:

Section I-C-1-a. All recombinant DNA research within the United States (U.S.) or its territories that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH, including research performed directly by the NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH Guidelines.

Section I-C-1-b. All recombinant DNA research performed abroad: Specifically:

Section I-C-1-b-(1). Research supported by NIH funds.

Section I-C-1-b-(2). If they involve testing in humans of materials containing recombinant DNA developed with NIH funds and if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b-(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the NIH Guidelines.

Section I-D. General Definitions

The following terms, which are used throughout the NIH Guidelines, are defined as follows:

Section I-D–1. An 'institution' is any public or private entity (including Federal, state, and local government agencies).

Section I-D–2. An 'Institutional Biosafety Committee' is a committee that: (i) meets the requirements for membership specified in Section IV-B–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B–2.

Section I-D–3. The 'Office of Recombinant DNA Activities (ORDA)' is the office within the NIH that is responsible for: (i) Reviewing and coordinating all activities relating to the NIH Guidelines, and (ii) performing other duties as defined in Section IV-C–3.

Section I-D–4. The 'Recombinant DNA Advisory Committee' is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C–2.

Section I-D–5. The 'NIH Director' is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I-D–6. 'Deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

B. Amendment to Section II, Containment, of the NIH Guidelines

The amended version of Section II reads as follows:

Section II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see Section V-A). The existing programs rely upon mechanisms that can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) The infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

Physical containment conditions within laboratories, described in Appendix G, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1–P through BL4–P) are described in Appendix P. BL1–P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2–P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3–P and BL4–P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1–P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1–P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2–P and BL3–P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4–P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1–N through BL4–N) are described in Appendix Q. BL1–N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2–N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3–N and BL4–N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the NIH Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the NIH Guidelines to permit the use of these procedures."

C. Amendment to Section III, Experiments Covered by the NIH Guidelines

The previous version of Section III-A–2 will be deleted as follows:

Section III-A–2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section III-A–2-a. Introduction conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendix L.

Section III-A–2-b. Deletion derivatives and single base changes not otherwise covered by the NIH Guidelines.

Section III-A–2-c. For extrachromosomal elements and microorganisms (including viruses), rearrangements and amplifications within a single genome. Rearrangements involving the introduction of DNA from different strains of the same species would not be covered by this exemption."

The amended version of Section III reads as follows:

Section III. Experiments Covered by the NIH Guidelines.

This section describes five categories of experiments involving recombinant DNA: (i) Those that require RAC review and NIH and Institutional Biosafety Committee approval before initiation (see Section III-A), (ii) those that require NIH/ORDA and Institutional Biosafety Committee approval before initiation (see Section III-B); (iii) those that require Institutional Biosafety Committee approval before initiation (see Section III-C), (iv) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-D), and (v) those that are exempt from the NIH Guidelines (see Section III-E).

Note: If an experiment falls into either Section III-A or Section III-B and one of the other categories, the rules pertaining to Section III-A or Section III-B shall be followed. If an experiment falls into Section III-E and into either Sections III-C or III-D categories as well, the experiment is considered exempt from the NIH Guidelines

Any change in containment level, which is different from those specified in the NIH Guidelines, may not be initiated without the express approval of NIH/ORDA (see Minor Actions, Section IV-C-1-b-(2) and its subsections).

Section III-A. Experiments That Require Institutional Biosafety Committee Approval, RAC Review, and NIH Approval Before Initiation

Experiments in this category are considered Major Actions (see Section IV-C-1-b-(1)) and cannot be initiated without submission of relevant information on the proposed experiment to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838, the publication of the proposal in the **Federal Register** for 15 days of comment, reviewed by the RAC, and specific approval by the NIH (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The containment conditions for such experiments will be recommended by the RAC and set by the NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D which may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section III-A-1. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

Section III-A-2. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V-U) shall be considered Major Actions (see Section IV-C-1-b-(1) and Appendix M-III), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. The requirement for RAC review shall not be considered to preempt any other required review or approval of experiments with one or more human subjects. Relevant Institutional Biosafety Committee and Institutional Review Board reviews and approvals of the proposal should be completed before submission to NIH. Certain experiments involving deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects may qualify for the Accelerated Review process (see Section III-B-2). Certain categories of experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and that are not covered by Section V-U, may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III-C-7).

Section III-B. Experiments That Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation

Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less Than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section III-B-1-(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/ORDA. The containment conditions for such experiments will be determined by NIH/ORDA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation (see Section IV-B-2-b-(1)).

Section III-B-2. Accelerated Review of Human Gene Transfer Experiments

As determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, certain categories of human gene transfer experiments may be considered as Minor Actions and qualify for Accelerated Review and approval (see Section IV-C-1-b-(2)-(a), Appendix M-III-A, and Appendix M-V). The RAC Chair will present a report of all NIH/ORDA approved human gene transfer protocols at the next regularly scheduled RAC meeting. If NIH/ORDA determines that an experiment does not qualify for the Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review ≥ 8 weeks prior to the next scheduled RAC meeting (See Section III-A-2).

Section III-B-3. Minor Modifications to Human Gene Transfer Experiments

A minor modification in a human gene transfer protocol is a modification that does not significantly alter the basic design of the protocol and that does not increase risk to human subjects or the environment. After approval has been obtained by the relevant Institutional Biosafety Committee and Institutional Review Board, NIH/ORDA will consider the change in consultation with the RAC Chair and one or more RAC members, as necessary. Submit minor modifications to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. The RAC Chair will provide a report on any such approvals at the next regularly scheduled RAC meeting.

Section III-C. Experiments That Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) The source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all

experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV–C–1–b–(2)–(c)).

Section III–C–1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (See Section V–A) as Host-Vector Systems

Section III–C–1–a. Experiments involving the introduction of recombinant DNA into Class 2 agents shall be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents shall be conducted with whole animals at BL2 or BL2–N (Animals) containment.

Section III–C–1–b. Experiments involving the introduction of recombinant DNA into Class 3 agents shall be conducted at BL3 containment. Experiments with such agents shall be conducted with whole animals at BL3 or BL3–N containment.

Section III–C–1–c. Experiments involving the introduction of recombinant DNA into Class 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment.

Section III–C–1–d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents shall be set on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T). Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment

Section III–C–2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (See Section V–A) is Cloned Into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III–C–2–a. Experiments in which DNA from Class 2 or Class 3 agents (see Section V–A) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III–E). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/ORDA approval (see Section III–B–1) or shall be conducted under NIH specified conditions as described in Appendix F.

Section III–C–2–b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/ORDA following a case-by-case review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T).

Section III–C–3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V–Q) being considered identical (see Section V–S), are considered defective and may be used in the absence of helper under the conditions specified in Section III–D–1.

Section III–C–3–a. Experiments involving the use of infectious or defective Class 2 animal viruses (see Section V–A, Appendix B–II, and Appendix B–II–E) in the presence of helper virus may be conducted at BL2.

Section III–C–3–b. Experiments involving the use of infectious or defective Class 3 animal viruses (see Section V–A and Appendix B–III–D) in the presence of helper virus may be conducted at BL3.

Section III–C–3–c. Experiments involving the use of infectious or defective Class 4 animal viruses (see Section V–A and Appendix B–IV–D) in the presence of helper virus may be conducted at BL4.

Section III–C–3–d. Experiments involving the use of infectious or defective Class 5 viruses (see Section V–A and Appendix B–V) in the presence of helper virus shall be determined on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T).

Section III–C–3–e. Experiments involving the use of infectious or defective animal or plant viruses in the presence of helper virus are not covered in Sections III–C–3–a through III–C–3–d and may be conducted at BL1.

Section III–C–4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may not be conducted at BL1–N containment. A minimum containment of BL2 or BL2–N is required.

Caution—Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III–C–4–a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study (see Section V–B).

Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III–C–4–b. For experiments involving recombinant DNA-modified Class 2, 3, 4, or 5 organisms, see Section V–A. It is important that the investigator

demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Section V–R and V–T).

Section III–C–4–b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III–C–1 or III–C–4–a, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III–C–5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III–C–5–a through III–C–5–e. If experiments involving whole plants are not described in Section III–C–5 and do not fall under Sections III–A, III–B, or III–E, they are included in Section III–D.

Note. For recombinant DNA experiments falling under Sections III–C–5–e through III–C–5–d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III–C–5–a. BL3–P (Plants) or BL2–P + biological containment is recommended for experiments involving most exotic (see Section V–W) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III–C–5–b. BL3–P or BL2–P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V–W) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

Section III–C–5–c. BL4–P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V–W) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III–C–5–d. BL3–P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight fall under Section III–B–1 and require NIH/ORDA and Institutional Biosafety Committee approval before initiation.

Section III–C–5–e. BL3–P or BL2–P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III–C–6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3–Large Scale.

Section III–C–7. Human Gene Transfer Experiments Not Covered by Sections III–A–2, III–B–2, III–B–3, and Not Considered Exempt Under Section V–U

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that are not covered by Sections III–A–2, III–B–2, III–B–3, and that are not considered exempt under Section V–U must be registered with NIH/ORDA. The relevant Institutional Biosafety Committee and Institutional Review Board must review and approve all experiments in this category prior to their initiation.

Section III–D. Experiments That Require Institutional Biosafety Committee Notice Simultaneous With Initiation

Experiments not included in Sections III–A, III–B, III–C, III–E, and their subsections are considered in Section III–D. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III–C) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV–A). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III–D and may be conducted at BL1 containment.

Section III–D–1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More Than Two-Thirds of the Genome of Any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V–Q) being considered identical (see Section V–S)) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III–C–3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III-D–2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-D-2-a. BL1–P is recommended for all experiments with recombinant DNA-containing plants and

plant-associated microorganisms not covered in Section III-D–2-b or other sections of the NIH Guidelines. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-W) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-D–2-b. BL2–P or BL1–P + biological containment is recommended for the following experiments:

Section III-D–2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-D–2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-W).

Section III-D–2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-D–2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious natural ecosystems (see Section V-W).

Section III-D–2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-E. Exempt Experiments

The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:

Section III-E–1. Those that are not in organisms or viruses.

Section III-E–2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III-E–3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III-E–4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-E–5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C–1-b-(1)-(c)). See Appendices A-I through A-VI for a list of natural exchangers that are exempt from the NIH Guidelines.

Section III-E–6. Those that do not present a significant risk to health or the environment (see Section IV-C–1-b-(1)-(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines."

D. Amendment to Section IV, Roles and Responsibilities of the NIH Guidelines

The amended version of Section IV-C–1-b reads as follows:

Section IV-C–1-b. Specific Responsibilities [NIH Director]

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the NIH Guidelines and presents no significant risk to health or the environment.

Section IV-C–1-b-(1). Major Actions

To execute Major Actions, the NIH Director shall seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and Proposed Actions to the NIH Guidelines shall be published in the **Federal Register** at least 15 days before the RAC meeting (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The NIH Director's decision, at his/her discretion, may be published in the **Federal Register** for 15 days of comment before final action is taken. The NIH Director's final decision, along with responses to public comments, shall be published in the **Federal Register**. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV-C–1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the NIH Guidelines when a Major Action is involved;

Section IV-C–1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the NIH Guidelines when a Major Action is involved;

Section IV–C–1–b–(1)–(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the NIH Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section IV–C–1–b–(1)–(d). Permitting experiments specified by Section III–A;

Section IV–C–1–b–(1)–(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I–II). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV–C–1–b–(1)–(f). Adopting other changes in the NIH Guidelines.

Section IV–C–1–b–(2). Minor Actions

NIH/ORDA shall carry out certain functions as delegated to it by the NIH Director (see Section IV–C–3). Minor Actions (as determined by NIH/ORDA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to the RAC and Institutional Biosafety Committee Chairs:

Section IV–C–1–b–(2)–(a). Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that qualify for the Accelerated Review process (see Section III–B–2);

Section IV–C–1–b–(2)–(b). Reviewing and approving minor changes to human gene transfer protocols under Section III–A–2 and III–B–2;

Section IV–C–1–b–(2)–(c). Changing containment levels for experiments that are specified in Section III;

Section IV–C–1–b–(2)–(d). Assigning containment levels for experiments not explicitly considered in the NIH Guidelines; and

Section IV–C–1–b–(2)–(e). Revising the Classification of Etiologic Agents for the purpose of these NIH Guidelines (see Section V–A).

Section IV–C–1–b–(2)–(f). Interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels;

Section IV–C–1–b–(2)–(g). Setting containment under Sections III–C–1–d and III–C–2–b;

Section IV–C–1–b–(2)–(h). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I–II);

Section IV–C–1–b–(2)–(i). Decertifying already certified host-vector systems;

Section IV–C–1–b–(2)–(j). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F); and

Section IV–C–1–b–(2)–(k). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–C–1–b–(2)–(c).

The amended version of Section IV–C–2 reads as follows:

Section IV–C–2. Recombinant DNA Advisory Committee (RAC). The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–C–1–b–(1).

The amended version of Section IV–C–3 reads as follows:

Section IV–C–3. Office of Recombinant DNA Activities (ORDA)

ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the NIH Director, including those authorities described in Section IV–C–1–b–(2). ORDA's responsibilities include, but are not limited to the following:

Section IV–C–3–a. Reviewing and approving experiments in conjunction with ad hoc experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD_{50} ≤100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K–12 (see Section III–B–1 and Appendices F–I and F–II);

Section IV–C–3–b. Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, in consultation with the RAC Chair and one or more RAC members, as necessary, that qualify for the Accelerated Review process (see Section III–B–2);

Section IV–C–3–c. Reviewing and approving minor changes to human gene transfer protocols approved under Sections III–A–2 and III–B–2, in consultation with the RAC Chair and one or more RAC members, as necessary;

Section IV–C–3–d. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV–B–2 will give its approval to the Institutional Biosafety Committee membership;

Section IV–C–3–e. Publishing in the Federal Register:

Section IV–C–3–e–(1). Announcements of RAC meetings and agendas at least 15 days in advance (Note—If the agenda for a RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least 72 hours in advance of the meeting);

Section IV–C–3–e–(2). Proposed Major Actions to the NIH Guidelines (see Section IV–C–1–b–(1)) at least 15 days prior to the RAC meeting;

Section IV–C–3–f. Serve as the focal point for data management of NIH-approved human gene transfer protocols approved under Sections III–A–2 and III–B–2 and registered with NIH/ORDA as required under Section III–C–7;

Section IV–C–3–g. Serve as the executive secretary of the RAC; and

Section IV–C–3–h. Maintain a list of Major and Minor Actions approved under Section III–A–2 and III–B–3 and a list of experiments registered with NIH/ORDA as described in Section III–C–7.

E. Amendment and Addition to Section V, Footnotes and References of Sections I–IV of the NIH Guidelines

The amended version of Section V–U reads as follows:

Section V–U. Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are not covered under Sections III–A–2, III–B–2, or III–B–3. Such studies may be initiated without RAC review and NIH approval if approved by another Federal agency."

The following new footnote, V–W is added to Section V:

Section V–W. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V–R). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

F. Addition to Appendix C–I, Recombinant DNA in Tissue Culture, of the NIH Guidelines

The amended version of Appendix C–I reads as follows:

Appendix C–I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family (see Appendix C–VI–D) being considered identical (see Appendix C–VI–E), that are propagated and maintained in cells in tissue culture are exempt from these NIH Guidelines with the exceptions listed in Appendix C–I–A.

Appendix C–I–A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and Institutional Biosafety Committee approval before initiation, (ii) experiments described in Section III–B which require NIH/ORDA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C–VI–A) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

G. Addition to Appendix G, Physical Containment, of the NIH Guidelines

Appendix G through G–I is amended to read as follows:

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level

1 through Biosafety Level 4. For large scale (over 10 liters) research or production, Appendix K supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3—Large Scale. For certain work with plants, Appendix P supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4—Plants. For certain work with animals, Appendix Q supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4—Animals.

Appendix G–I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G–III–A through G–III–J). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV–B–1–e and IV–B–4–d). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV–B–4–d and IV–B–4–e). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV–B–1–f).

The Laboratory Safety Monograph (see Appendix G–III–O) and Biosafety in Microbiological and Biomedical Laboratories (see Appendix G–III–B) describe practices, equipment, and facilities in detail.

H. Addition of Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants, to the NIH Guidelines

The following new appendix, Appendix P, reads as follows:

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

Appendix P shall supersede Appendix G when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) Are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P–I. General Plant Biosafety Levels

Appendix P–I–A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

Appendix P–I–B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix P–I–C. Four biosafety levels, referred to as Biosafety Level (BL) 1—Plants (P), BL2–P, BL3–P, and BL4–P, are established in Section II. The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in Section III. These biosafety levels are described in Appendix P–II. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P–I–D. BL1–P through BL4–P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in Appendix P–III, provide flexible approaches to ensure the safe conduct of research.

Appendix P–I–E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix P–III), if botanical reproductive structures are produced that have the potential of being released.

Appendix P–II. Physical Containment Levels

Appendix P–II–A. Biosafety Level 1—Plants (BL1–P)

Appendix P–II–A–1. Standard Practices (BL1–P)

Appendix P–II–A–1–a. Greenhouse Access (BL1–P)

Appendix P–II–A–1–a–(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P–II–A–1–a–(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1–P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P-II-A–1-b. Records (BL1–P)

Appendix P-II-A–1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-A–1-c. Decontamination and Inactivation (BL1–P)

Appendix P-II-A–1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-A–1-d. Control of Undesired Species and Motile Macroorganisms (BL1–P)

Appendix P-II-A–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-A–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-A–1-e. Concurrent Experiments Conducted in the Greenhouse (BL1–P)

Appendix P-II-A–1-e-(1). Experiments involving other organisms that require a containment level lower than BL1–P may be conducted in the greenhouse concurrently with experiments that require BL1–P containment, provided that all work is conducted in accordance with BL1–P greenhouse practices.

Appendix P-II-A–2. Facilities (BL1–P)

Appendix P-II-A–2-a. Definitions (BL1–P)

Appendix P-II-A–2-a-(1). The term 'greenhouse' refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-A–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P-II-A–2-b. Greenhouse Design (BL1–P)

Appendix P-II-A–2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P-II-A–2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix P-II-B. Biosafety Level 2—Plants (BL2–P)

Appendix P-II-B–1. Standard Practices (BL2–P)

Appendix P-II-B–1-a. Greenhouse Access (BL2–P)

Appendix P-II-B–1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix P-II-B–1-a-(2). Personnel shall be required to read and follow instructions on BL2–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-B–1-b. Records (BL2–P)

Appendix P-II-B–1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-B–1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-B–1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-B–1-c. Decontamination and Inactivation (BL2–P)

Appendix P-II-B–1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-B–1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P-II-B–1-d. Control of Undesired Species and Motile Macroorganisms (BL2–P)

Appendix P-II-B–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-B–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-B–1-e. Concurrent Experiments Conducted in the Greenhouse (BL2–P)

Appendix P-II-B–1-e-(1). Experiments involving other organisms that require a containment level lower than BL2–P may be conducted in the greenhouse concurrently with experiments that require BL2–P containment provided that all work is conducted in accordance with BL2–P greenhouse practices.

Appendix P-II-B–1-f. Signs (BL2–P)

Appendix P-II-B–1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) The plants in use, and (iii) any special requirements for using the area.

Appendix P-II-B–1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-B–1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-B–1-g. Transfer of Materials (BL2–P)

Appendix P-II-B–1-g-(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix P-II-B–1-h. Greenhouse Practices Manual (BL2–P)

Appendix P-II-B–1-h-(1). A greenhouse practices manual shall be

prepared or adopted. This manual shall: (i) Advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P-II-B–2. Facilities (BL2–P)

Appendix P-II-B–2-a. Definitions (BL2–P)

Appendix P-II-B–2-a-(1). The term 'greenhouse' refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-B–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix P-II-B–2-b. Greenhouse Design (BL2–P)

Appendix P-II-B–2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P-II-B–2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P-II-B–2-c. Autoclaves (BL2–P)

Appendix P-II-B–2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P-II-B–2-d. Supply and Exhaust Air Ventilation Systems (BL2–P)

Appendix P-II-B–2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P-II-B–2-e. Other (BL2–P)

Appendix P-II-B–2-e-(1). BL2–P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Appendix P-II-C. Biosafety Level 3—Plants (BL3–P)

Appendix P-II-C–1. Standard Practices (BL3–P)

Appendix P-II-C–1-a. Greenhouse Access (BL3–P)

Appendix P-II-C–1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P-II-C–1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-C–1-b. Records (BL3–P)

Appendix P-II-C–1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-C–1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-C–1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable).

Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-C–1-c. Decontamination and Inactivation (BL3–P)

Appendix P-II-C–1-c-(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P-II-C–1-d. Control of Undesired Species and Motile Macroorganisms (BL3–P)

Appendix P-II-C–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-C–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-C–1-e. Concurrent Experiments Conducted in the Greenhouse (BL3–P)

Appendix P-II-C–1-e-(1). Experiments involving organisms that require a containment level lower than BL3–P may be conducted in the greenhouse concurrently with experiments that require BL3–P containment provided that all work is conducted in accordance with BL3–P greenhouse practices.

Appendix P-II-C–1-f. Signs (BL3–P)

Appendix P-II-C–1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-C–1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-C–1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-C–1-g. Transfer of Materials (BL3–P)

Appendix P-II-C–1-g-(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface

of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P-II-C–1-h. Greenhouse Practices Manual (BL3–P)

Appendix P-II-C–1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) Advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Appendix P-II-C–1-i. Protective Clothing (BL3–P)

Appendix P-II-C–1-i-(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P-II-C–1-i-(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P-II-C–1-j. Other (BL3–P)

Appendix P-II-C–1-j-(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P-II-C–1-j-(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P-II-C–2. Facilities (BL3–P)

Appendix P-II-C–2-a. Definitions (BL3–P)

Appendix P-II-C–2-a-(1). The term 'greenhouse' refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-C–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P-II-C–2-b. Greenhouse Design (BL3–P)

Appendix P-II-C–2-b-(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P-II-C–2-b-(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-C–2-b-(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P-II-C–2-b-(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-C–2-b-(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-C–2-b-(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-C–2-b-(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P-II-C–2-c. Autoclaves (BL3–P)

Appendix P-II-C–2-c-(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P-II-C–2-d. Supply and Exhaust Air Ventilation Systems (BL3–P)

Appendix P-II-C–2-d-(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P-II-C–2-d-(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80–85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52- 68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P–II–C–2–e. Other (BL3–P)

Appendix P–II–C–2–e–(1). BL3–P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P–II–C–2–e–(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P–II–D. Biosafety Level 4—Plants (BL4–P)

Appendix P–II–D–1. Standard Practices (BL4–P)

Appendix P–II–D–1–a. Greenhouse Access (BL4–P)

Appendix P–II–D–1–a–(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P–II–D–1–a–(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P–II–D–1–a–(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the

greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P–II–D–1–a–(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P–II–D–1–a–(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4–P practices and procedures.

Appendix P–II–D–1–b. Records (BL4–P)

Appendix P–II–D–1–b–(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P–II–D–1–b–(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–D–1–b–(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P–II–D–1–b–(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P–II–D–1–c. Decontamination and Inactivation (BL4–P)

Appendix P–II–D–1–c–(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P–II–D–1–c–(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P–II–D–1–c–(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P–II–D–1–d. Control of Undesired Species and Motile Macroorganisms (BL4–P)

Appendix P–II–D–1–d–(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P–II–D–1–d–(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4–P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P–II–D–1–e. Concurrent Experiments Conducted in the Greenhouse (BL4–P)

Appendix P–II–D–1–e–(1). Experiments involving organisms that require a containment level lower than BL4–P may be conducted in the greenhouse concurrently with experiments that require BL4–P containment provided that all work is conducted in accordance with BL4–P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4–P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P–II–D–1–f. Signs (BL4–P)

Appendix P–II–D–1–f–(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P–II–D–1–f–(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

Appendix P–II–D–1–f–(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P–II–D–1–g. Transfer of Materials (BL4–P)

Appendix P–II–D–1–g–(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P–II–D–g–(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P–II–D–1–h. Greenhouse Practices Manual (BL4–P)

Appendix P–II–D–1–h–(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

Appendix P–II–D–1–i. Protective Clothing (BL4–P)

Appendix P–II–D–1–i–(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P–II–D–1–i–(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P–II–D–1–i–(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P–II–D–2. Facilities (BL4–P)

Appendix P–II–D–2–a. Greenhouse Design (BL4–P)

Appendix P–II–D–2–a–(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure

should be considered when constructing or renovating the greenhouse facility.

Appendix P–II–D–2–a–(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P–II–D–2–a–(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P–II–D–2–a–(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P–II–D–2–a–(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P–II–D–2–a–(6). The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P–II–D–2–a–(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P–II–D–2–a–(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P–II–D–2–b. Autoclaves (BL4–P)

Appendix P–II–D–2–b–(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P–II–D–2–c. Supply and Exhaust Air Ventilation Systems (BL4–P)

Appendix P–II–D–2–c–(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P–II–D–2–c–(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P–II–D–2–d. Other (BL4–P)

Appendix P–II–D–2–d–(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P–II–D–2–d–(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P–II–D–2–d–(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P–II–D–2–d–(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P–III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P–III–A. Biological Containment Practices (Plants)

Appendix P–III–A–1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P-III-B. Biological Containment Practices (Microorganisms)

Appendix P-III-B–1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible

to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P-III-C. Biological Containment Practices (Macroorganisms)

Appendix P-III-C–1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) Use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

I. Addition of Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals, to the NIH Guidelines

The following new appendix, Appendix Q, reads as follows:

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

Appendix Q-I. General Considerations

Appendix Q-I-A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in Section III. For the purpose of animal research, four levels of containment are established. These are referred to as BL1–Animals (N), BL2–N, BL3–N, and BL4–N and are described in the following sections of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

Appendix Q-I-B. Disposal of Animals (BL1–N through BL4–N)

Appendix Q-I-B–1. When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q-I-B–2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Appendix Q-II. Physical and Biological Containment Levels

Appendix Q-II-A. Biosafety Level 1 - Animals (BL1–N)

Appendix Q-II-A–1. Standard Practices (BL1–N)

Appendix Q-II-A–1-a. Animal Facility Access (BL1–N)

Appendix Q-II-A–1-a-(1). The containment area shall be locked.

Appendix Q-II-A–1-a-(2). Access to the containment area shall be limited or restricted when experimental animals are being held.

Appendix Q-II-A–1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-A–1-b. Other (BL1–N)

Appendix Q-II-A–1-b-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non- transgenic animals.

Appendix Q-II-A–1-b-(2) A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-A–1-b-(3). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-A–2. Animal Facilities (BL1–N)

Appendix Q-II-A–2-(a). Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix Q-II-B. Biosafety Level 2 - Animals (BL2–N) (see Appendix Q-III-A)

Appendix Q-II-B–1. Standard Practices (BL2–N)

Appendix Q-II-B–1-a. Animal Facility Access (BL2–N)

Appendix Q-II-B–1-a-(1). The containment area shall be locked.

Appendix Q-II-B–1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-B–1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-B–1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Appendix Q-II-B–1-a-(5). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-B–1-b. Decontamination and Inactivation (BL2–N)

Appendix Q-II-B–1-b-(1). Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

Appendix Q-II-B–1-b-(2). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B–1-c. Signs (BL2–N)

Appendix Q-II-B–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-B–1-d. Protective Clothing (BL2–N)

Appendix Q-II-B–1-d-(1). Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

Appendix Q-II-B–1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-B–1-e. Records (BL2–N)

Appendix Q-II-B–1-e-(1). Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-B–1-e-(2). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Appendix Q-II-B–1-f. Transfer of Materials (BL2–N)

Appendix Q-II-B–1-f-(1). Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-B–1-g. Other (BL2–N)

Appendix Q-II-B–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-B–1-g-(2). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B–1-g-(3). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Appendix Q-II-B–1-g-(4). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-B–1-g-(5). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-B–1-g-(6). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-B–1-g-(7). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-B–1-g-(8). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-B–2. Animal Facilities (BL2–N)

Appendix Q-II-B–2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-B–2-b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix Q-II-B–2-c. The animal containment area shall be designed so that it can be easily cleaned.

Appendix Q-II-B–2-d. Windows that open shall be fitted with fly screens.

Appendix Q-II-B–2-e. An autoclave shall be available for decontamination of laboratory wastes.

Appendix Q-II-B–2-f. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Appendix Q-II-C. Biosafety Level 3—Animals (BL3–N) (See Appendix Q-III-B)

Appendix Q-II-C–1. Standard Practices (BL3–N)

Appendix Q-II-C–1-a. Animal Facility Access (BL3–N)

Appendix Q-II-C–1-a-(1). The containment area shall be locked.

Appendix Q-II-C–1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-C–1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-C–1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

Appendix Q-II-C–1-a-(5). Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Appendix Q-II-C–1-b. Decontamination and Inactivation (BL3–N)

Appendix Q-II-C–1-b-(1). The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-C–1-b-(2). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C–1-b-(3). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C–1-b-(4). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q-II-C–1-b-(5). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C–1-c. Signs (BL3–N)

Appendix Q-II-C–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-C–1-d. Protective Clothing (BL3–N)

Appendix Q-II-C–1-d-(1). Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3–N area and wearing of personal clothing.

Appendix Q-II-C–1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-C–1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-C–1-e. Records (BL3–N)

Appendix Q-II-C–1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-C–1-e-(2). Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-C–1-e-(3). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Appendix Q-II-C–1-f. Transfer of Materials (BL3–N)

Appendix Q-II-C–1-f-(1). Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-C–1-f-(2). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q-II-C–1-g. Other (BL3–N)

Appendix Q-II-C–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among nontransgenic animals.

Appendix Q-II-C–1-g-(2). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Appendix Q-II-C–1-g-(3). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-C-1-g-(4). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-C-1-g-(5). Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

Appendix Q-II-C-1-g-(6). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-C-1-g-(7). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-C-1-g-(8). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-C-1-g-(9). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-C-1-g-(10). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-g-(11). Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

Appendix Q-II-C-1-g-(12). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-C-1-g-(13). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-g-(14). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-C-2. Animal Facilities (BL3-N)

Appendix Q-II-C-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-C-2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-C-2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Appendix Q-II-C-2-d. An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-C-2-e. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Appendix Q-II-C-2-f. Access doors to the containment area shall be self-closing.

Appendix Q-II-C-2-g. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

Appendix Q-II-C-2-h. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C-2-i. An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-C-2-j. If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

Appendix Q-II-C-2-k. Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-C-2-l. In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Appendix Q-II-C-2-m. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q-II-C-2-n. Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

Appendix Q-II-D. Biosafety Level 4—Animals (BL4-N) (See Appendix Q-III-C)

Appendix Q-II-D-1. Standard Practices (BL4-N)

Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)

Appendix Q-II-D-1-a-(1). Individuals under 16 years of age shall not be permitted to enter the animal area.

Appendix Q–II–D–1–a–(2). The containment area shall be locked.

Appendix Q–II–D–1–a–(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–D–1–a–(4). The containment building shall be controlled and have a locking access.

Appendix Q–II–D–1–a–(5). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

Appendix Q–II–D–1–a–(6). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

Appendix Q–II–D–1–a–(7). Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

Appendix Q–II–D–1–a–(8). Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Appendix Q–II–D–1–b. Decontamination and Inactivation (BL4–N)

Appendix Q–II–D–1–b–(1). All contaminated liquid or solid wastes shall be decontaminated before disposal.

Appendix Q–II–D–1–b–(2). The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q–II–D–1–b–(3). All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

Appendix Q–II–D–1–b–(4). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated.

Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D–1-b-(5). When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D–1-b-(6). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D–1-b-(7). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

Appendix Q-II-D–1-b-(8). An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D–1-b-(9). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D–1-c. Signs (BL4–N)

Appendix Q-II-D–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-D–1-d. Protective Clothing (BL4–N)

Appendix Q-II-D–1-d-(1). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4–N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

Appendix Q-II-D–1-d-(2). A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D–1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-D–1-e. Records (BL4–N)

Appendix Q-II-D–1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-D–1-e-(2). A system shall be established for: (i) Reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be

provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-D–1-e-(3). When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix Q-II-D–1-e-(4). A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Appendix Q-II-D–1-f. Transfer of Materials (BL4–N)

Appendix Q-II-D–1-f-(1). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D–1-f-(2). Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4–N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4–N animal facility to one with a lower containment classification.

Appendix Q-II-D–1-f-(3). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Appendix Q-II-D–1-g. Other (BL4–N)

Appendix Q-II-D–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-D–1-g-(2). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-D–1-g-(3). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-D–1-g-(4). Experiments involving other organisms that require containment levels lower than BL4–N may be conducted in the same area concurrently with experiments requiring BL4–N containment provided that they are conducted in accordance with BL4–N practices.

Appendix Q-II-D–1-g-(5). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-D–1-g-(6). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-D–1-g-(7). A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

Appendix Q-II-D–1-g-(8). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-D–1-g-(9). The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

Appendix Q-II-D–1-g-(10). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D–1-g-(11). An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Appendix Q-II-D–1-g-(12). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-D–1-g-(13). Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-D–2. Animal Facilities (BL4–N)

Appendix Q-II-D–2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

Appendix Q-II-D–2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-D–2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

Appendix Q-II-D–2-d. An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D–2-e. Access doors to the containment area shall be self-closing.

Appendix Q-II-D–2-f. All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

Appendix Q-II-D–2-g. The BL4–N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

Appendix Q-II-D–2-h. A necropsy room shall be provided within the BL4–N containment area.

Appendix Q-II-D–2-i. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide.

The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D–2-j. A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-D–2-k. Exhaust air from BL4–N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4–N containment area.

Appendix Q-II-D–2-l. All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1×10^{-3} micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80–120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

Appendix Q-II-D–2-m. If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1×10^{8} organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

Appendix Q-II-D–2-n. All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

Appendix Q-II-D–2-o. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q–II–D–2–p. Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

Appendix Q–II–D–2–q. The supply water distribution system shall be fitted with a back-flow preventer or break tank.

Appendix Q–II–D–2–r. All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

Appendix Q–II–D–2–s. Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

Appendix Q–III. Footnotes and References for Appendix Q

Appendix Q–III–A. If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

Appendix Q–III–B. Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3–N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

Appendix Q–III–C. Classes 4 and 5 microorganisms pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import Class 5 agents, special approval must be obtained from U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

Appendix Q–III–D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely

by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1–P through BL4–P containment practices and procedures as specified in Appendices G and P.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

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Harold Varmus,

Director, National Institutes of Health.

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National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines); Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

June 1994.

These NIH Guidelines supersede all earlier versions and shall be in effect until further notice.

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Section I. Scope of the NIH Guidelines

Section I–A. Purpose

The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) Recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I–A–1. Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, has been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval (see exceptions in Sections I–A–2 and I–A–3).

Section I–A–2. Certain experiments that involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) shall be considered Major Actions (see Section IV–C–1–b–(1)), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review (see Section III–A–2).

Section I–A–3. Experiments involving the transfer of recombinant DNA to one or more human subjects that are not considered under Section III–A–2 may qualify for Accelerated Review (see Section III–B–2 and Appendix M–V) and will be considered as Minor Actions (see Section IV–C–1–b–(2)–(a)). Actions that qualify for Accelerated Review will be reviewed and approved by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary.

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III–C–7).

Section I–B. Definition of Recombinant DNA Molecules

In the context of the NIH Guidelines, recombinant DNA molecules are defined as either: (i) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

Section I–C. General Applicability

Section I–C–1. The NIH Guidelines are applicable to:

Section I–C–1–a. All recombinant DNA research within the United States (U.S.) or its territories that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH, including research performed directly by the NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH Guidelines.

Section I–C–1–b. All recombinant DNA research performed abroad: Specifically:

Section I–C–1–b–(1). Research supported by NIH funds.

Section I–C–1–b–(2). If they involve testing in humans of materials containing recombinant DNA developed with NIH funds and if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I–C–1–b–(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the NIH Guidelines.

Section I–D. General Definitions

The following terms, which are used throughout the NIH Guidelines, are defined as follows:

Section I–D–1. An "institution" is any public or private entity (including Federal, state, and local government agencies).

Section I–D–2. An "Institutional Biosafety Committee" is a committee that: (i) Meets the requirements for membership specified in Section IV–B–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV–B–2.

Section I–D–3. The "Office of Recombinant DNA Activities (ORDA)"

is the office within the NIH that is responsible for: (i) Reviewing and coordinating all activities relating to the NIH Guidelines, and (ii) performing other duties as defined in Section IV–C–3.

Section I–D–4. The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV–C–2.

Section I–D–5. The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I–D–6. "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see section V–A). The existing programs rely upon mechanisms that can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) The infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

Physical containment conditions within laboratories, described in Appendix G, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1–P through BL4–P) are described in Appendix P. BL1–P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2–P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3–P and BL4–P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1–P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1–P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2–P and BL3–P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4–P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1–N through BL4–N) are described in Appendix Q. BL1–N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2–N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3–N and BL4–N

describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the NIH Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the NIH Guidelines to permit the use of these procedures.

Section III. Experiments Covered by the NIH Guidelines

This section describes five categories of experiments involving recombinant DNA: (i) Those that require RAC review and NIH and Institutional Biosafety Committee approval before initiation (see section III–A), (ii) those that require NIH/ORDA and Institutional Biosafety Committee approval before initiation (see section III–B); (iii) those that require Institutional Biosafety Committee approval before initiation (see section III–C), (iv) those that require Institutional Biosafety Committee notification simultaneous with initiation (see section III–D), and (v) those that are exempt from the NIH Guidelines (see section III–E).

Note: If an experiment falls into either section III–A or section III–B and one of the other categories, the rules pertaining to section III–A or section III–B shall be followed. If an experiment falls into section III–E and into either sections III–C or III–D categories as well, the experiment is considered exempt from the NIH Guidelines.

Any change in containment level, which is different from those specified in the NIH Guidelines, may not be initiated without the express approval of NIH/ORDA (see Minor Actions, section IV–C–1–b–(2) and its subsections).

Section III–A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Approval Before Initiation

Experiments in this category are considered Major Actions (see section IV–C–1–b–(1)) and cannot be initiated without submission of relevant information on the proposed experiment to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838, the publication of the proposal in the Federal Register for 15 days of comment, reviewed by the RAC, and specific approval by the NIH (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M–VI). The containment conditions for such experiments will be recommended by the RAC and set by the NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D which may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section III–A–1. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see section V–B), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

Section III–A–2. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see section V–U) shall be considered Major Actions (see section IV–C–1–b–(1) and Appendix M–III), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. The requirement for RAC review shall not be considered to preempt any other required review or approval of experiments with one or more human subjects. Relevant Institutional Biosafety Committee and Institutional Review Board reviews and approvals of the proposal should be completed before submission to NIH. Certain experiments involving deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects may qualify for the Accelerated Review process (see section III–B–2). Certain categories of experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and that are not covered by section V–U, may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see section III–C–7).

Section III–B. Experiments That Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation

Section III–B–1. Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K–12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section III–B–1–(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/ORDA. The containment conditions for such experiments will be determined by NIH/ORDA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation (see section IV–B–2–b–(1)).

Section III–B–2. Accelerated Review of Human Gene Transfer Experiments

As determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, certain categories of human gene transfer experiments may be considered as Minor Actions and qualify for Accelerated Review and approval (see section IV–C–1–b–(2)–(a), Appendix M–III–A, and Appendix M–V). The RAC Chair will present a report of all NIH/ORDA approved human gene transfer protocols at the next regularly scheduled RAC meeting. If NIH/ORDA determines that an experiment does not qualify for the Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review ≥ 8 weeks prior to the next scheduled RAC meeting (See section III–A–2).

Section III-B–3. Minor Modifications to Human Gene Transfer Experiments

A minor modification in a human gene transfer protocol is a modification that does not significantly alter the basic design of the protocol and that does not increase risk to human subjects or the environment. After approval has been obtained by the relevant Institutional Biosafety Committee and Institutional Review Board, NIH/ORDA will consider the change in consultation with the RAC Chair and one or more RAC members, as necessary. Submit minor modifications to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. The RAC Chair will provide a report on any such approvals at the next regularly scheduled RAC meeting.

Section III-C. Experiments that Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) The source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C–1-b-(2)-(c)).

Section III-C–1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (see Section V-A) as Host-Vector Systems

Section III-C–1-a. Experiments involving the introduction of recombinant DNA into Class 2 agents shall be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents shall be conducted with whole animals at BL2 or BL2–N (Animals) containment.

Section III-C–1-b. Experiments involving the introduction of recombinant DNA into Class 3 agents shall be conducted at BL3 containment. Experiments with such agents shall be conducted with whole animals at BL3 or BL3–N containment.

Section III-C–1-c. Experiments involving the introduction of recombinant DNA into Class 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment.

Section III-C–1-d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents shall be set on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T). Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment.

Section III-C–2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (see Section V-A) is Cloned Into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III-C–2-a. Experiments in which DNA from Class 2 or Class 3 agents (see Section V-A) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III-E). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/ORDA approval (see Section III-B–1) or shall be conducted under NIH specified conditions as described in Appendix F.

Section III-C–2-b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/ORDA following a case-by-case review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T).

Section III-C–3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-Q) being considered identical (see Section V-S), are considered defective and may be used in the absence of helper under the conditions specified in Section III-D–1.

Section III-C–3-a. Experiments involving the use of infectious or defective Class 2 animal viruses (see Section V-A, Appendix B-II, and Appendix B-II-E) in the presence of helper virus may be conducted at BL2.

Section III-C–3-b. Experiments involving the use of infectious or defective Class 3 animal viruses (see Section V-A and Appendix B-III-D) in the presence of helper virus may be conducted at BL3.

Section III-C–3-c. Experiments involving the use of infectious or defective Class 4 animal viruses (see Section V-A and Appendix B-IV-D) in the presence of helper virus may be conducted at BL4.

Section III-C–3-d. Experiments involving the use of infectious or defective Class 5 viruses (see Section V-A and Appendix B-V) in the presence of helper virus shall be determined on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T).

Section III-C–3-e. Experiments involving the use of infectious or defective animal or plant viruses in the presence of helper virus are not covered in Sections III-C–3-a through III-C–3-d and may be conducted at BL1.

Section III-C–4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into

the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may not be conducted at BL1–N containment. A minimum containment of BL2 or BL2–N is required.

Caution—Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-C–4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study (see Section V-B). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-C–4-b. For experiments involving recombinant DNA-modified Class 2, 3, 4, or 5 organisms, see Section V-A. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Section V-R and V-T).

Section III-C–4-b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-C–1 or III-C–4-a, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-C–5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III-C–5-a through III-C–5-e. If experiments involving whole plants are not described in Section III-C–5 and do not fall under Sections III-A, III-B, or III-E, they are included in Section III-D.

Note: For recombinant DNA experiments falling under Sections III-C–5-a through III-C–5-d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III-C–5-a. BL3–P (Plants) or BL2–P + biological containment is recommended for experiments involving most exotic (see Section V-W) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III-C–5-b. BL3–P or BL2–P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V-W) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

Section III-C–5-c. BL4–P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V-W) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III-C–5-d. BL3–P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight fall under Section III-B–1 and require NIH/ORDA and Institutional Biosafety Committee approval before initiation.

Section III-C–5-e. BL3–P or BL2–P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III-C–6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3–Large Scale.

Section III-C–7. Human Gene Transfer Experiments Not Covered by Sections III-A-2, III-B–2, III-B–3, and Not Considered Exempt Under Section V-U

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that are not covered by Sections III-A–2, III-B–2, III-B–3, and that are not considered exempt under Section V-U must be registered with NIH/ORDA. The relevant Institutional Biosafety Committee and Institutional Review Board must review and approve all experiments in this category prior to their initiation.

Section III-D. Experiments that Require Institutional Biosafety Committee Notice Simultaneous With Initiation

Experiments not included in Sections III-A, III-B, III-C, III-E, and their subsections are considered in Section III-D. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III-C) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV-A). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-D and may be conducted at BL1 containment.

Section III-D–1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of Any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-Q) being considered identical (see Section V-S)) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-C–3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III-D–2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-D–2-a. BL1–P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in Section III-D–2-b or other sections of the NIH Guidelines. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-W) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-D–2-b. BL2–P or BL1–P + biological containment is recommended for the following experiments:

Section III-D–2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-D–2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-W).

Section III-D–2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-D–2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious natural ecosystems (see Section V-W).

Section III–D–2–b–(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V–W).

Section III–E. Exempt Experiments

The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:

Section III–E–1. Those that are not in organisms or viruses.

Section III–E–2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III–E–3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III–E–4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III–E–5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV–C–1–b–(1)–(c)). See Appendices A–I through A–VI for a list of natural exchangers that are exempt from the NIH Guidelines.

Section III–E–6. Those that do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines.

Section IV, Roles and Responsibilities

Section IV–A. Policy

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The NIH Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The NIH Guidelines are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and Principal Investigator in determining safeguards that should be implemented. The NIH Guidelines will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of the NIH Guidelines as well as to their specifics. Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the NIH Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by the NIH as necessary.

Section IV–B. Responsibilities of the Institution

Section IV-B–1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the NIH Guidelines

is responsible for ensuring that the research is conducted in full conformity with the provisions of the NIH Guidelines. In order to fulfill this responsibility, the institution shall:

Section IV-B–1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the NIH Guidelines. As part of its general responsibilities for implementing the NIH Guidelines, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the NIH Guidelines. Such procedures may include: (i) Statements formulated by the institution for the general implementation of the NIH Guidelines, and (ii) any additional precautionary steps the institution deems appropriate.

Section IV-B–1-b. Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B–2-a and carries out the functions detailed in

Section IV-B–2-b.

Section IV-B–1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) Conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in Section IV-B–3.

Section IV-B–1-d. Assist and ensure compliance with the NIH Guidelines by Principal Investigators conducting research at the institution as specified in

Section IV-B–4.

Section IV-B–1-e. Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the NIH Guidelines. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

Section IV-B–1-f. Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The Laboratory Safety Monograph discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the Laboratory Safety Monograph include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the Laboratory Safety Monograph are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section IV-B–1-g. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to NIH/ORDA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section IV-B–2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

Section IV-B–2-a. Membership and Procedures

Section IV-B–2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3 or BL4, a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B–3).

Section IV-B–2-a-(2). In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B–2-a-(3). The institution shall file a report with NIH/ORDA which includes the names and biographical sketches of all Institutional Biosafety Committee members, including community members, in such form and at such times as required by NIH/ORDA.

Section IV-B–2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in

which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B–2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B–2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B–2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section IV-B–2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B–2-b-(1). Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the NIH Guidelines as specified in Section III and approving those research projects that are found to conform with the NIH Guidelines. This review shall include: (i) independent assessment of the containment levels required by the NIH Guidelines for the proposed research, and (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research.

Section IV-B–2-b-(2). Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B–2-b-(3). Lowering containment levels for certain experiments as specified in Section III-C–2-a.

Section IV-B–2-b-(4). Setting containment levels as specified in Sections III-C–4-b and III-C–5.

Section IV-B–2-b-(5). Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the NIH Guidelines.

Section IV-B–2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

Note: The Laboratory Safety Monograph describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the Laboratory Safety Monograph. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B–2-b-(7). Reporting any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/ORDA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

Section IV-B–2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the NIH Guidelines until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B–2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B–2.

Section IV-B–3. Biological Safety Officer (BSO)

Section IV-B–3-a. The institution shall appoint a Biological Safety Officer if it engages in large scale research or production activities involving viable organisms containing recombinant DNA molecules.

Section IV-B–3-b. The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section IV-B–3-c. The Biological Safety Officer's duties include, but are not be limited to:

Section IV-B–3-c-(1). Periodic inspections to ensure that laboratory standards are rigorously followed;

Section IV-B–3-c-(2). Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

Section IV-B–3-c-(3). Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

Section IV-B–3-c-(4). Providing advice on laboratory security;

Section IV-B–3-c-(5). Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

Note: See the Laboratory Safety Monograph for additional information on the duties of the Biological Safety Officer.

Section IV–B–4. Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research.

Section IV–B–4–a. General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

Section IV–B–4–a–(1). Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections III–A, III–B, and III–C) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the NIH Guidelines;

Section IV–B–4–a–(2). Determine whether experiments are covered by Section III–D and that the appropriate procedures are followed;

Section IV–B–4–a–(3). Report any significant problems, violations of the NIH Guidelines, or any significant research–related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) within 30 days (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838);

Section IV–B–4–a–(4). Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH/ORDA (reports to NIH/ORDA shall be sent to the Office of

Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838);

Section IV–B–4–a–(5). Be adequately trained in good microbiological techniques;

Section IV–B–4–a–(6). Adhere to Institutional Biosafety Committee–approved emergency plans for handling accidental spills and personnel contamination; and

Section IV–B–4–a–(7). Comply with shipping requirements for recombinant DNA molecules (see Appendix H for shipping requirements and the Laboratory Safety Monograph for technical recommendations).

Section IV–B–4–b. Submissions by the Principal Investigator to the NIH/ORDA

The Principal Investigator shall:

Section IV–B–4–b–(1). Submit information to NIH/ORDA for certification of new host–vector systems;

Section IV–B–4–b–(2). Petition NIH/ORDA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the NIH Guidelines;

Section IV–B–4–b–(3). Petition NIH/ORDA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in Sections III–A and III–B of the NIH Guidelines;

Section IV–B–4–b–(4). Petition NIH/ORDA for determination of containment for experiments requiring case–by–case review; and

Section IV–B–4–b–(5). Petition NIH/ORDA for determination of containment for experiments not covered by the NIH Guidelines.

Section IV–B–4–c. Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

Section IV–B–4–c–(1). Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines;

Section IV–B–4–c–(2). Select appropriate microbiological practices and laboratory techniques to be used for the research;

Section IV–B–4–c–(3). Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host–vector system), if covered under

Sections III–A, III–B, III–C, or III–D, to the Institutional Biosafety Committee for review and approval or disapproval; and

Section IV–B–4–c–(4). Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Section IV–B–4–d. Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

Section IV–B–4–d–(1). Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

Section IV–B–4–d–(2). Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

Section IV–B–4–d–(3). Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Section IV–B–4–e. Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

Section IV–B–4–e–(1). Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

Section IV–B–4–e–(2). Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), the Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) (reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838);

Section IV–B–4–e–(3). Correct work errors and conditions that may result in the release of recombinant DNA materials; and

Section IV–B–4–e–(4). Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Section IV–C. Responsibilities of the National Institutes of Health (NIH)

Section IV–C–1. NIH Director

The NIH Director is responsible for: (i) establishing the NIH Guidelines, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the NIH Guidelines that involve ORDA and the RAC. ORDA's responsibilities under the NIH Guidelines are administrative. Advice from the RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

Section IV–C–1–a. General Responsibilities

The NIH Director is responsible for:

Section IV–C–1–a–(1). Promulgating requirements as necessary to implement the NIH Guidelines;

Section IV–C–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–C–2 (RAC membership is specified in its charter and in Section IV–C–2); and

Section IV–C–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–C–3.

Section IV–C–1–b. Specific Responsibilities

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the NIH Guidelines and presents no significant risk to health or the environment.

Section IV–C–1–b–(1). Major Actions

To execute Major Actions, the NIH Director shall seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and Proposed Actions to the NIH Guidelines shall be published in the **Federal Register** at least 15 days before the RAC meeting (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M–VI). The NIH Director's decision, at his/her discretion, may be published in the **Federal Register** for 15 days of comment before final action is taken. The NIH Director's final decision, along with responses to public comments, shall be published in the **Federal Register**. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV–C–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the NIH Guidelines when a Major Action is involved;

Section IV–C–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the NIH Guidelines when a Major Action is involved;

Section IV–C–1–b–(1)–(c). Promulgating and amending a list of classes of recombinant DNA molecules

to be exempt from the NIH Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section IV–C–1–b–(1)–(d). Permitting experiments specified by Section III–A;

Section IV–C–1–b–(1)–(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I–II). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV–C–1–b–(1)–(f): Adopting other changes in the NIH Guidelines.

Section IV–C–1–b–(2). Minor Actions

NIH/ORDA shall carry out certain functions as delegated to it by the NIH Director (see Section IV–C–3). Minor Actions (as determined by NIH/ORDA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to the RAC and Institutional Biosafety Committee Chairs:

Section IV–C–1–b–(2)–(a). Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that qualify for the Accelerated Review process (see Section III–B–2);

Section IV–C–1–b–(2)–(b). Reviewing and approving minor changes to human gene transfer protocols under Section III–A–2 and III–B–2;

Section IV–C–1–b–(2)–(c). Changing containment levels for experiments that are specified in Section III;

Section IV–C–1–b–(2)–(d). Assigning containment levels for experiments not explicitly considered in the NIH Guidelines; and

Section IV–C–1–b–(2)–(e). Revising the Classification of Etiologic Agents for the purpose of these NIH Guidelines (see Section V–A).

Section IV–C–1–b–(2)–(f). Interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels;

Section IV–C–1–b–(2)–(g). Setting containment under Sections III–C–1–d and III–C–2– b;

Section IV–C–1–b–(2)–(h). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I–II);

Section IV–C–1–b–(2)–(i). Decertifying already certified host-vector systems;

Section IV-C–1-b-(2)-(j). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F); and

Section IV-C–1-b-(2)-(k). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV-C–1-b-(2)-(c).

Section IV-C–1-b-(3). The NIH Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

Section IV-C–2. Recombinant DNA Advisory Committee (RAC)

The RAC is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the DHHS Secretary, the DHHS Assistant Secretary for Health, and the NIH Director. The RAC consists of 25 members including the Chair, appointed by the DHHS Secretary or his/her designee, at least fourteen of whom are selected from authorities knowledgeable in the fields of molecular genetics, molecular biology, recombinant DNA research, or other scientific fields. At least six members of the RAC shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as non-voting members.

Nominations for the RAC may be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

All meetings of the RAC shall be announced in the **Federal Register**, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a Major Action under Section IV-C–1-b-(1) may be added to an agenda following **Federal Register** publication.

The RAC shall be responsible for advising the NIH Director on the actions listed in Sections IV-C–1-b-(1).

Section IV-C–3. Office of Recombinant DNA Activities (ORDA)

ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the NIH Director, including those authorities described in Section IV-C–1-b-(2). ORDA's responsibilities include, but are not limited to the following:

Section IV-C–3-a. Reviewing and approving experiments in conjunction with ad hoc experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an $LD_{50} \leq 100$ nanograms per kilogram body weight in organisms other than Escherichia coli K–12 (see Section III-B–1 and Appendices F-I and F-II);

Section IV-C–3-b. Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, in consultation with the RAC Chair and one or more RAC members, as necessary, that qualify for the Accelerated Review process (see Section III-B–2);

Section IV-C–3-c. Reviewing and approving minor changes to human gene transfer protocols approved under Sections III-A–2 and III-B–2, in consultation with the RAC Chair and one or more RAC members, as necessary;

Section IV-C–3-d. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV-B–2 will give its approval to the Institutional Biosafety Committee membership;

Section IV-C–3-e. Publishing in the **Federal Register**:

Section IV–C–3–e–(1). Announcements of RAC meetings and agendas at least 15 days in advance (NOTE—If the agenda for a RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least 72 hours in advance of the meeting);

Section IV–C–3–e–(2). Proposed Major Actions to the NIH Guidelines (see Section IV–C–1–b–(1)) at least 15 days prior to the RAC meeting;

Section IV–C–3–f. Serve as the focal point for data management of NIH-approved human gene transfer protocols approved under Sections III–A–2 and III–B–2 and registered with NIH/ORDA as required under Section III–C–7;

Section IV–C–3–g. Serve as the executive secretary of the RAC; and

Section IV–C–3–h. Maintain a list of Major and Minor Actions approved under Section III–A–2 and III–B–3 and a list of experiments registered with NIH/ORDA as described in Section III–C–7.

Section IV–C–4. Other NIH Components

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

Section IV–D. Compliance with the NIH Guidelines

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the NIH Guidelines. The policies on noncompliance are as follows:

All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in: (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Information concerning noncompliance with the NIH Guidelines may be brought forward by any person. It should be delivered to both NIH/ORDA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the NIH Guidelines, applicable DHHS and Public Health Service procedures shall govern.

Section IV–E. Voluntary Compliance

Section IV–E–1. Basic Policy

Individuals, corporations, and institutions not otherwise covered by the NIH Guidelines are encouraged to do so by following the standards and procedures set forth in Sections I through IV. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the NIH Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the NIH Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in Sections IV–E–2 through IV–E–5–b in order to address these concerns.

Section IV–E–2. Institutional Biosafety Committee Approval

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the NIH Guidelines may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. Section IV–B–2–a–(4) is modified to that extent for purposes of these institutions.

Section IV–E–3. Certification of Host-Vector Systems

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section IV–E–5-a will be issued only after consultation with the institution as to the content of the notice.

Section IV–E–4. Requests for Exemptions and Approvals

Requests for exemptions or other approvals as required by the NIH Guidelines should be submitted based on the procedures set forth in Sections I through IV. In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section IV–E–5-a will be issued only after consultation with the institution as to the content of the notice.

Section IV–E–5. Protection of Proprietary Data

Section IV–E–5-a. General

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential." Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes—of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the RAC are "special Government employees."

In submitting to NIH for purposes of voluntary compliance with the NIH Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised except to the extent that earlier release in the judgment of the NIH Director is necessary to protect against an imminent hazard to the public or the environment.

Section IV–E–5-b. Presubmission Review

Any institution not otherwise covered by the NIH Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for presubmission review should be submitted to NIH/ORDA along with the records involved to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. NIH/ORDA will seek a determination from the responsible official under DHHS regulations (45 Code of Federal Regulations, Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/ORDA files, will be considered records of the institution and not NIH/ORDA, and will not be received as part of NIH/ORDA files. No copies will be made of such records.

NIH/ORDA will inform the institution of the DHHS Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/ORDA files. If the institution instructs NIH/ORDA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or ORDA. The DHHS Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act if at the time of the determination the records were in NIH/ORDA files and a request was received for such files under the Freedom of Information Act.

Section V. Footnotes and References of Sections I Through IV

Section V–A. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Services, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333. The NIH Director, with advice of the RAC, may revise the classification for the purposes of the NIH Guidelines (see Section IV–C–1-b-(2)-(e)). The revised list of organisms in each class is reprinted in Appendix B.

Section V–B. Section III describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines" (see Section IV–B–4-c-(1)). For cases falling under Sections III–A through III–D, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the NIH Guidelines for the proposed research" (see Section IV–B–2-b-(1)). The Institutional Biosafety Committee may refer specific cases to NIH/ORDA as part of NIH/ORDA's functions to "provide advice to all within and outside NIH" (see Section IV–C–3). NIH/ORDA may request advice from the RAC as part of the RAC's responsibility for "interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels" (see Section IV–C–1-b-(2)-(f)).

Section V–C. Laboratory Safety at the Centers for Disease Control, September 1974, U.S. Department of Health, Education, and Welfare Publication No. CDC 75–8118.

Section V–D. Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

Section V–E. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, October 1974, U.S. Department of Health, Education, and Welfare, Publication No. (NIH) 75–790.

Section V–F. National Institutes of Health Biohazards Safety Guide, 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740–00383.

Section V–G. A. Hellman, M. N. Oxman, and R. Pollack (eds.), 1973, Biohazards in Biological Research, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Section V–H. Furr, A. K., Handbook of Laboratory Safety, 2nd ed, The Chemical Rubber Co., Boca Raton, Florida, 1990.

Section V–I. American Public Health Association, Bodily, J. L., General Administration of the Laboratory, 6th ed., "Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections," New York, 1981.

Section V–J. H. M. Darlow, Safety in the Microbiological Laboratory, in J. R. Norris and D. W. Robbins (eds.), Methods in Microbiology, Academic Press, Inc, New York, New York, 1969, pp. 169–204.

Section V–K. C. M. Collins, E. G. Hartley, and R. Pilsworth, The Prevention of Laboratory Acquired Infection, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Section V–L. Chatigny, M. A, "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W.W. Umbreit (ed.), Advances in Applied Microbiology, Academic Press, New York, New York, 1961, 3:131–192.

Section V–M. Design Criteria for Viral Oncology Research Facilities, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, DHEW Publication No. (NIH) 75–891, 1975.

Section V–N. Kuehne, R. W., Biological Containment Facility for Studying Infectious Disease, Appl. Microbiol. 26:239–243, 1973.

Section V–O. Runkle, R. B., and G. B. Phillips, Microbial Containment Control Facilities, Van Nostrand Reinhold, New York, 1969.

Section V–P. Chatigny, M. A., and D. I. Clinger, "Contamination Control in Aerobiology," in R. L. Dimmick and A. B. Akers (eds.), An Introduction to Experimental Aerobiology, John Wiley & Sons, New York, 1969, pp. 194–263.

Section V–Q. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews (ed.), Intervirology 12 (129–296), 1979.

Section V–R. A U.S. Department of Agriculture permit is required for the importation, interstate movement, and release into the environment of certain organisms that are plant or animal pathogens, whether genetically engineered or not. Permits are required for veterinary biologics and for certain plants or microorganisms derived through genetic engineering using genetic sequences from plant pests (pathogens). Specific information about regulated organisms and procedures for obtaining a permit for regulated organisms may be obtained from the Director, Biotechnology, Biologics, and Environmental Protection, Animal and

Plant Health Inspection Service, U.S. Department of Agriculture, 6505 Belcrest Road, Room 850, Hyattsville, Maryland 20782, (301) 436–7601.

Section V–S. i.e., the total of all genomes within a family shall not exceed two-thirds of the genome.

Section V–T. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

Section V–U. Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are not covered under Sections III–A–2, III–B–2, or III–B–3. Such studies may be initiated without RAC review and NIH approval if approved by another Federal agency.

Section V–V. For recombinant DNA experiments in which the intent is to modify stably the genome of cells of one or more human subjects (see Sections III–A–2, III–B–2, and III–B–3).

Section V–W. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V–R). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

Appendix A. Exemptions Under Section III–E–5—Sublists of Natural Exchangers

Certain specified recombinant DNA molecules that "consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from these NIH Guidelines (see Section III–E–5). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see Section IV–C–1–b–(1)–(c)). See Appendices A–I through A–VI for a list of natural exchangers that are exempt from the NIH Guidelines." Section III–E–5 describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see Classification of Bergey's Manual of Determinative Bacteriology; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control, Atlanta, Georgia, and NIH Office of Biosafety, Bethesda, Maryland).

Appendix A–I. Sublist A

Genus *Escherichia*
Genus *Shigella*
Genus *Salmonella*—including Arizona
Genus *Enterobacter*
Genus *Citrobacter*—including *Levinea*
Genus *Klebsiella*—including *oxytoca*
Genus *Erwinia*
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, and Pseudomonas mendocina
Serratia marcescens
Yersinia enterocolitica

Appendix A–II. Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

Appendix A–III. Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Appendix A–IV. Sublist D

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Appendix A–V. Sublist E

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

Appendix A–VI. Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

Appendix B. Classification of Etiologic Agents and Oncogenic Viruses on the Basis of Hazard (See Appendix B–VI–A).

Appendix B–I. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes shall be considered Class 1 agents.

Appendix B–II. Class 2 Agents

Appendix B–II–A. Class 2 Bacterial Agents

Acinetobacter calcoaceticus
Actinobacillus—all species
Aeromonas hydrophila
Amycolata autotrophica
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
Haemophilus ducreyi, H. influenzae
Klebsiella—all species except oxytoca
Legionella pneumophila
Leptospira interrogans—all serotypes
Listeria—all species
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycobacterium avium
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 5
Neisseria gonorrhoea, N. meningitides
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Pasteurella—all species except those listed in Class 3
Rhodococcus equi
Salmonella—all species and all serotypes
Shigella-all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae, S. pyogenes
Treponema carateum, T. pallidum, and T. pertenue

Vibrio cholerae, V. parahemolyticus
Yersinia enterocolitica

Appendix B-II-B. Class 2 Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

Appendix B-II-C. Class 2 Parasitic Agents

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxocara canis
Toxoplasma gondii
Trichinella spiralis
Trypanosoma cruzi

Appendix B-II-D. Class 2 Viral, Rickettsial, and Chlamydial Agents

Adenoviruses—human—all types
Cache Valley virus
Coronaviruses
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis viruses—associated antigen material
Herpesviruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses —all types, wild and attenuated
Poxviruses—all types except Alastrim, Smallpox, and Whitepox which are Class 5 and Monkey pox which depending on experiments is in Class 3 or Class 4
Rabies virus—all strains except Rabies street virus which should be classified in Class 3
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae* (Monkey B virus) and Marburg virus which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vesicular stomatitis virus (see Appendix B-VI-B)
Vole rickettsia
Yellow fever virus, 17D vaccine strain

Appendix B-II-E. Class 2 Oncogenic Viruses (See Appendix B-VI-C)

Appendix B-II-E-1. Low-Risk Oncogenic Viruses

Adenovirus 7–Simian virus 40 (Ad7–SV40)
Adenovirus
Avian leukosis virus
Bovine leukemia virus
Bovine papilloma virus
Chick-embryo-lethal orphan (CELO) virus or fowl adenovirus 1
Dog sarcoma virus
Guinea pig herpes virus
Lucke (Frog) virus
Hamster leukemia virus
Marek's disease virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Polyoma virus
Rat leukemia virus
Rous sarcoma virus
Shope fibroma virus
Shope papilloma virus
Simian virus 40 (SV–40)

Appendix B-II-E-2. Moderate-Risk Oncogenic Viruses

Adenovirus 2–Simian virus 40 (Ad2–SV40)
Epstein-Barr virus (EBV)
Feline leukemia virus (FeLV)
Feline sarcoma virus (FeSV)
Gibbon leukemia virus (GaLV)
Herpesvirus (HV) *ateles*
Herpesvirus (HV) *saimiri*
Simian sarcoma virus (SSV)–1
Yaba

Appendix B–III. Class 3 Agents

Appendix B–III–A. Class 3 Bacterial Agents

Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium bovis, M. tuberculosis
Pasteurella multocide type—"buffalo" and other foreign virulent strains (see Appendix B–VI–B)
Pseudomonas mallei (see Appendix B–VI–B)
Pseudomonas pseudomallei (see Appendix B–VI–B)
Yersinia pestis

Appendix B–III–B. Class 3 Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

Appendix B–III–C. Class 3 Parasitic Agents

None

Appendix B–III–D. Class 3 Viral, Rickettsial, and Chlamydial Agents

Monkey pox virus—when used *in vitro* (see Appendix B–VI–D)
Arboviruses—all strains except those in Class 2 and 4. (Arboviruses indigenous to the United States are in Class 3 except those listed in Class 2. West Nile and Semliki Forest viruses may be classified up or down depending on the conditions of use and geographical location of the laboratory).
Dengue virus—when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used *in vitro*

Appendix B–IV. Class 4 Agents

Appendix B–IV–A. Class 4 Bacterial Agents

None

Appendix B–IV–B. Class 4 Fungal Agents

None

Appendix B–IV–C. Class 4 Parasitic Agents

None

Appendix B–IV–D. Class 4 Viral, Rickettsial, and Chlamydial Agents

Ebola fever virus
Monkey pox virus—when used for transmission or animal inoculation experiments (see Appendix B–VI–D)
Hemorrhagic fever agents—including Crimean hemorrhagic fever, (Congo), Junin, and Machupo viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex—including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Venezuelan equine encephalitis virus, epidemic strains—when used for transmission or animal inoculation experiments
Yellow fever virus-wild—when used for transmission or animal inoculation experiments

Appendix B–V. Class 5 Agents (see Appendix B–VI–E)

Appendix B–V–A. Animal Disease Organisms which are Forbidden Entry into the United States by Law

Foot and mouth disease virus

Appendix B–V–B. Animal Disease Organisms and Vectors which are Forbidden Entry into the United States by U.S. Department of Agriculture Policy

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Mycoplasma mycoides—contagious bovine pleuropneumonia
Mycoplasma agalactiae—contagious agalactia of sheep
Nairobi sheep disease virus
Newcastle disease virus—Asiatic strains
Rhinderpest virus
Rickettsia ruminatium—heart water
Rift valley fever virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Theileria parva—East Coast fever
Trypanosoma evansi
Trypanosoma vivax—Nagana
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Appendix B–V–C. Organisms which may not be Studied in the United States Except at Specified Facilities

Alastrim (see Appendix B–VI–D)
Small pox (see Appendix B–VI–D)
White pox (see Appendix B–VI–D)

Appendix B–VI. Footnotes and References of Appendix B

Appendix B–VI–A. The original reference for this classification was the publication Classification of Etiologic Agents on the Basis of Hazard, 4th edition, July 1974, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these NIH Guidelines, this list has been revised by the NIH.

Appendix B–VI–B. A U.S. Department of Agriculture permit, required for import and interstate transport of pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products Office, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Appendix B–VI–C. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790, October 1974.

Appendix B–VI–D. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

Appendix B–VI–E. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C. Exemptions Under Section III–E–6

Section III–E–6 states that exempt from these NIH Guidelines are "those that do not present a significant risk to health or the environment (see Section IV–C–1–b–(1)–(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines." The following classes of experiments are exempt under Section III–E–6:

Appendix C–I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family (see Appendix C–VI–D) being considered identical (see Appendix C–VI–E), that are propagated and maintained in cells in tissue culture are exempt from these NIH Guidelines with the exceptions listed in Appendix C–I–A.

Appendix C–I–A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III–A which require specific RAC review and NIH and Institutional Biosafety Committee approval before initiation, (ii) experiments described in Section III–B which require NIH/ORDA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C–VI–A) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Appendix C–II. *Escherichia coli* K–12 Host-Vector Systems

Experiments which use *Escherichia coli* K–12 host-vector systems, with the exception of those experiments listed in Appendix C–II–A, are exempt from the NIH Guidelines provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C–VI–B) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K–12 of DNA from prokaryotes that exchange genetic information (see Appendix C–VI–C) with Escherichia coli may be performed with any Escherichia coli K–12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K–12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C–II–A. Exceptions

The following categories of experiments are not exempt from the NIH Guidelines: (i) experiments described in Section III–A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III–B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C–VI–A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-III. Saccharomyces Host-Vector Systems

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the NIH Guidelines. For these exempt experiments, BL1 physical containment is recommended. For large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-IV. Bacillus subtilis or Bacillus licheniformis Host-Vector Systems

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-IV-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these NIH Guidelines.

Bacillus amyloliquefaciens
Bacillus amylosaccharíticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicúm
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylcoccus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Appendix C-V-A. Exceptions

The following categories of experiments are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require Institutional Biosafety Committee, specific RAC review, and NIH approval before initiation, (ii) experiments described in Section III–B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C–VI–A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C–VI. Footnotes and References of Appendix C

Appendix C–VI–A. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333.

Appendix C–VI–A–1. The NIH Director, with advice of the RAC, may revise the classification for the purposes of these NIH Guidelines (see Section IV–C–1–b–(2)–(d)). The revised list of organisms in each class is reprinted in Appendix B.

Appendix C–VI–B. A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

Appendix C–VI–C. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III–E–5.

Appendix C–VI–D. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R.E.F. Matthews (ed.), Intervirology 12 (129–296); 1979.

Appendix C–VI–E. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

Appendix D. Major Actions Taken Under the NIH Guidelines

Under Section IV–C–1–b–(1), the NIH Director may take certain actions with regard to the NIH Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix E. Certified Host-Vector Systems (see Appendix I)

While many experiments using *Escherichia coli* K–12, *Saccharomyces cerevisiae*, and *Bacillus subtilis* are currently exempt from the NIH Guidelines under Section III–E, some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems follows:

Appendix E–I. Bacillus subtilis

Appendix E–I–A. Bacillus subtilis Host-Vector 1 Systems

The following plasmids are accepted as the vector components of certified *B. subtilis* systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of Host-Vector 1 systems based on these plasmids.

Appendix E–I–B. Bacillus Subtilis Host-Vector 2 Systems

The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E–II. Saccharomyces Cerevisiae

Appendix E–II–A. Saccharomyces Cerevisiae Host-Vector 2 Systems

The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III. Escherichia coli

Appendix E-III-A. *Escherichia coli* (EK2) Plasmid Systems

The *Escherichia coli* K–12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following *Escherichia coli*/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in *Escherichia coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: YIpI, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEP24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III-B. *Escherichia coli* (EK2) Bacteriophage Systems

The following are certified EK2 systems based on bacteriophage lambda:

Vector	Host
λgt *WES*λB′	DP50*supF*
λgt *WES*λB°	DP50*supF*
λgt *ZJ* virλB′	*Escherichia coli* K–12
λgtALO•λB	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Escherichia coli K–12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50*supF* provided that the su-strain not be used as a propagation host.

Appendix E-IV. Neurospora crassa

Appendix E-IV-A. Neurospora crassa Host-Vector 1 Systems

The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion: In1 (inositolless) strains 37102, 37401, 46316, 64001, and 89601. Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

Appendix E–V. Streptomyces

Appendix E–V–A. *Streptomyces* Host-Vector 1 Systems

The following *Streptomyces* species: *Streptomyces coelicolor, S. lividans, S. parvulus*, and *S. griseus*. The following are accepted as vector components of certified *Streptomyces* Host-Vector 1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

Appendix E–VI. Pseudomonas Putida

Appendix E–VI–A. Pseudomonas putida Host-Vector 1 Systems

Pseudomonas putida strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates

Appendix F–I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of <100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under Section III–B–1 and require Institutional Biosafety Committee and NIH/ORDA approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} ≥100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an LD_{50} of <100 micrograms per kilograms and >100 nanograms per kilograms body weight require Institutional Biosafety Committee approval and registration with NIH/ORDA prior to initiating the experiments. A list of toxin molecules classified as to LD_{50} is available from NIH/ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. The results of such tests shall be forwarded to NIH/ORDA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see Section IV–C–1–b–(2)–(e)).

Appendix F–II. Cloning of Toxin Molecule Genes in Escherichia coli K–12

Appendix F–II–A. Cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under Biosafety Level (BL) 2 + EK2 or BL3 + EK1 containment conditions.

Appendix F–II–B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an LD_{50} of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordetella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F–II–C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *Escherichia coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *Escherichia coli* and of *Yersinia enterocolitica*.

Appendix F–III. Cloning of Toxic Molecule Genes in Organisms Other Than Escherichia coli K–12

Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight in host-vector systems other than *Escherichia coli* K–12 will be evaluated by NIH/ORDA in consultation with *ad hoc* toxin experts (see Sections III–B–1 and IV–C–1–b–(2)–(e)).

Appendix F–IV. Specific Approvals

An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix G. Physical Containment

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large scale (over 10 liters) research or production, Appendix K supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3—Large Scale. For certain work with plants, Appendix P supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4—Plants. For certain work with animals, Appendix Q supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4—Animals.

Appendix G–I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G–III–A through G–III–J). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV–B–1–e and IV–B–4–d). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV–B–4–d and IV–B–4–e). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV–B–1–f).

The Laboratory Safety Monograph (see Appendix G–III–O) and Biosafety in Microbiological and Biomedical Laboratories (see Appendix G–III–B) describe practices, equipment, and facilities in detail.

Appendix G–II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see Appendix G–III–B). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see Appendix G–III–C).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The NIH Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given by the NIH Director, with the advice of the RAC to other combinations which achieve an equivalent level of containment (see Section IV-C–1-b-(2)-(c)).

Appendix G–II–A. Biosafety Level 1 (BL1) (see Appendix G–III–M)

Appendix G–II–A–1. Standard Microbiological Practices (BL1).

Appendix G–II–A–1-a. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

Appendix G–II–A–1-b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G–II–A–1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G–II–A–1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G–II–A–1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G–II–A–1-f. Persons wash their hands: (i) After they handle materials involving organisms containing recombinant DNA molecules

and animals, and (ii) before exiting the laboratory.

Appendix G-II-A–1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-A–1-h. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Appendix G-II-A–2. Special Practices (BL1). Appendix G-II-A–2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-A–2-b. An insect and rodent control program is in effect.

Appendix G-II-A–3. Containment Equipment (BL1). Appendix G-II-A–3-a. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Appendix G-II-A–4. Laboratory Facilities (BL1). Appendix G-II-A–4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-A–4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-A–4-c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-A–4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-A–4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B. Biosafety Level 2 (BL2) (see Appendix G-III-N)

Appendix G-II-B–1. Standard Microbiological Practices (BL2). Appendix G-II-B–1-a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

Appendix G-II-B–1-b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-B–1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-B–1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-B–1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-B–1-f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

Appendix G-II-B–1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-B–1-h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B–2. Special Practices (BL2). Appendix G-II-B–2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-B–2-b. The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-B–2-c. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Appendix G-II-B–2-d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G-II-B–2-e. An insect and rodent control program is in effect.

Appendix G-II-B–2-f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G-II-B–2-g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G-II-B–2-h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G-II-B–2-i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-B–2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

Appendix G-II-B–2-k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/ORDA. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G-II-B–2-l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G-II-B–2-m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B–3. Containment Equipment (BL 2). Appendix G-II-B–3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B–3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of

materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL 2). Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

Appendix G-II-C. Biosafety Level 3 (BL3) (see Appendix G-III-P)

Appendix G-II-C-1. Standard Microbiological Practices (BL3). Appendix G-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-C-1-d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G-II-C-1-e. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Appendix G-II-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G-II-C-2. Special Practices (BL3) Appendix G-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.

Appendix G-II-C-2-b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-C-2-c. The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-C-2-d. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

Appendix G-II-C-2-e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Appendix G-II-C-2-f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G-II-C-2-h. An insect and rodent program is in effect.

Appendix G-II-C-2-i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Appendix G-II-C-2-j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G-II-C-2-k. Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see Appendix G-III-K), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G-II-C-2-n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-C-2-o. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix G-II-C-2-p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms

containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/ORDA. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix G–II–C–2–r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix G–II–C–2–s. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

Appendix G–II–C–2–t. Alternative Selection of Containment Equipment (BL3) Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Appendix G–Table 1.

Appendix G–II–C–3. Containment Equipment (BL3). Appendix G–II–C–3–a. Biological safety cabinets (Class I, II, or III) (see Appendix G–III–L) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Appendix G–II–C–4. Laboratory Facilities (BL3) Appendix G–II–C–4–a. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix G–II–C–4–b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area. Appendix G–II–C–4–c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–C–4–d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–C–4–e. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix G–II–C–4–f. Windows in the laboratory are closed and sealed.

Appendix G–II–C–4–g. Access doors to the laboratory or containment module are self-closing.

Appendix G–II–C–4–h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix G–II–C–4–i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Appendix G–II–C–4–j. The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G–III–L)) that avoids any interference with the air balance of the cabinets or building exhaust system.

Appendix G–II–D. Biosafety Level 4 (BL4)

Appendix G–II–D–1. Standard Microbiological Practices (BL4)

Appendix G–II–D–1–a. Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

Appendix G–II–D–1–b. Only mechanical pipetting devices are used.

Appendix G–II–D–1–c. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

Appendix G–II–D–1–d. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G–II–D–2. Special Practices (BL4). Appendix G–II–D–2–a. Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Appendix G–II–D–2–b. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix G–II–D–2–c. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other persons responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel

indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Appendix G–II–D–2–d. Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

Appendix G–II–D–2–e. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.

Appendix G–II–D–2–f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G–II–D–2–g. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Appendix G–II–D–2–h. An insect and rodent control program is in effect.

Appendix G–II–D–2–i. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix G–II–D–2–j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

Appendix G–II–D–2–k. A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/ORDA. Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G–II–D–2–l. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see Appendix G–III–K), open cages placed in ventilated enclosures, or solid-wall and- bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G–II–D–2–m. Alternative Selection of Containment Equipment (BL4)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G—Table 1.

Appendix G–II–D–3. Containment Equipment (BL4). Appendix G–II–D–3–a. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G–II–D–4. Laboratory Facilities (BL4). Appendix G–II–D–4–a. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G–II–D–4–b. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

Appendix G–II–D–4–c. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G–II–D–4–d. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G–II–D–4–e. Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G–II–D–4–f. A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

Appendix G–II–D–4–g. If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

Appendix G–II–D–4–h. If water fountains are provided, they are foot operated and are located in the facility

corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

Appendix G–II–D–4–i. Access doors to the laboratory are self-closing and locking.

Appendix G–II–D–4–j. Any windows are breakage resistant.

Appendix G–II–D–4–k. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Appendix G–II–D–4–l. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Appendix G–II–D–4–m. Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

Appendix G–II–D–4–n. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

Appendix G–II–D–4–o. The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

Appendix G–II–D–4–p. The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system. If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G–III–L)) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G–II–D–4–q. A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

APPENDIX G—TABLE 1.—POSSIBLE ALTERNATIVE COMBINATIONS OF PHYSICAL AND BIOLOGICAL CONTAINMENT SAFEGUARDS

Classification of physical & biological containment	Alternate physical containment			Alternate biological containment
	Laboratory facilities	Laboratory practices	Laboratory equipment	
BL3/HV2	BL3	BL3	BL3	HV2
	BL3	BL3	BL4	HV1
BL3/HV1	BL3	BL3	BL3	HV1
	BL3	BL3	BL2	HV2
BL4/HV1	BL4	BL4	BL4	HV1
	BL4	BL4	BL3	HV2

BL—Biosafety Level.
HV—Host-Vector System.

Appendix G–III. Footnotes and References of Appendix G

Appendix G–III–A. Laboratory Safety at the Center for Disease Control, U.S. Department of Health, Education, and Welfare Publication No. CDC 75–8118, September 1974.

Appendix G–III–B. Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

Appendix G–III–C. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790, October 1974.

Appendix G–III–D. National Institutes of Health Biohazards Safety Guide, U.S.

Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740–00383, 1974.

Appendix G–III–E. A. Hellman, M. N. Oxman, and R. Pollack (eds.), Biohazards in Biological Research, Cold Spring Harbor Laboratory 1973.

Appendix G–III–F. N. V. Steere (ed.), Handbook of Laboratory Safety, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

Appendix G–III–G. Bodily, J. L, "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections, American Public Health Association, New York, 1970, pp. 11–28.

Appendix G–III–H. Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), Methods in Microbiology, Academic Press, Inc., New York, pp. 169–204.

Appendix G–III–I. The Prevention of Laboratory Acquired Infection, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Appendix G–III–J. Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), Advances in Applied Microbiology, Academic Press, New York, New York, 1961, 3:131–192.

Appendix G–III–K. Horsfall, F. L. Jr., and J. H. Baner, Individual Isolation of Infected Animals in a Single Room, J. Bact., 1940, 40, 569–580.

Appendix G–III–L. Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

Appendix G–III–M. Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see Appendix G–III–B).

Appendix G–III–N. Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see Appendix G–III–B).

Appendix G–III–O. Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research, NIH, Bethesda, Maryland 1978.

Appendix G–III–P. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.

Appendix H. Shipment

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171–179).

Appendix H–I. Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

Appendix H–II. Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: (i) the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal

pathogen or a plant pest; or (ii) the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

Appendix H–III. Footnotes and References of Appendix H

For further information on shipping etiologic agents contact: (i) The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639–3883, FTS 236–3883; (ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366–4545; or (iii) U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782; for Animal Pathogens call (301) 436–7885; for Plant Pests (301) 436–6799.

Appendix I. Biological Containment (See Appendix E)

Appendix I–I. Levels of Biological Containment

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I–I–A through I–II–B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

Appendix I–I–A. Host-Vector 1 Systems

Host-Vector 1 systems provide a moderate level of containment. Specific Host-Vector 1 systems are:

Appendix I–I–A–1. Escherichia coli K–12 Host-Vector 1 Systems (EK1). The host is always *Escherichia coli* K–12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (see Appendices I–III–A through G) and variants of bacteriophage, such as lambda (see Appendices I–III–H through O). The *Escherichia coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

Appendix I–I–A–2. Other Host-Vector 1 Systems. At a minimum, hosts and vectors shall be comparable in containment to *Escherichia coli* K–12 with a non-conjugative plasmid or bacteriophage vector. Appendix I–II describes the data to be considered and mechanism for approval of Host-Vector 1 systems.

Appendix I–I–B. Host-Vector 2 Systems

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be $<1/10^8$ under specified conditions. Specific Host-Vector 2 systems are:

Appendix I–I–B–1. For *Escherichia coli* K–12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than $1/10^8$ host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

Appendix I–I–B–2. For Escherichia coli K–12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than $1/10^8$ phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

Appendix I–II. Certification of Host-Vector Systems

Appendix I–II–A. Responsibility. Host-Vector 1 systems (other than Escherichia coli K–12) and Host-Vector 2 systems may not be designated as such until they have been certified by the NIH Director. Requests for certification of host-vector systems may be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Proposed host-vector systems will be reviewed by the RAC (see section IV-C-1-b-(1)-(e)). Initial review will based on the construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more RAC members and/or *ad hoc* experts. The RAC will evaluate the subcommittee's report and any other available information at the next scheduled RAC meeting. The NIH Director is responsible for certification of host-vector systems, following advice of the RAC. Minor modifications to existing host-vector systems (i.e., those that are of minimal or no consequence to the properties relevant to containment), may be certified by the NIH Director without prior RAC review (see section IV-C-1-b-(2)-(h)). Once a host-vector system has been certified by the NIH Director, a notice of certification will be sent by NIH/ORDA to the applicant and to the Institutional Biosafety Committee Chairs. A list of all currently certified host-vector systems is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838. The NIH Director may rescind the certification of a host-vector system (see section IV-C-1-b-(2)-(i)). If certification is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher level of physical containment level, unless NIH determines that the already constructed clones incorporate adequate biological containment. Certification of an host-vector system does not extend to modifications of either the host or vector component of that system. Such modified systems shall be independently certified by the NIH Director. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications to a certified host-vector system requires submission of complete testing data.

Appendix I–II–B. Data To Be Submitted for Certification

Appendix I–II–B–1. Host-Vector 1 Systems Other than Escherichia coli K–12. The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) a description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and the type of information is exchanged; and any relevant information about its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an Host-Vector 1 system.

Appendix I–II–B–2. Host-Vector 2 Systems. Investigators planning to request Host-Vector 2 systems certification may obtain instructions from NIH/ORDA concerning data to be submitted (see Appendices I–III–N and O). In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals or one or more human subjects. Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments. Data shall be submitted 12 weeks prior to the RAC meeting at which such data will be considered by the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed Host Vector 2 systems prior to consideration of the system by the RAC and its subcommittee. Specific instructions concerning the submission of data for proposed *Escherichia coli* K–12 Host-Vector 2 system (EK2) involving either plasmids or bacteriophage in *Escherichia coli* K–12, are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

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Appendix I–III–L. Donoghue, D.J., and P.A. Sharp, An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance, Gene, 1977, 1, pp. 209–227.

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Appendix J. Biotechnology Research Subcommittee

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

Membership of the Biotechnology Research Subcommittee will include Federal agencies that support biotechnology research. Agencies represented are: U.S. Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Interior.

Department of Justice, Department of State, Department of Veterans Affairs, Agency for International Development, Environmental Protection Agency, National Aeronautics and Space Administration, and National Science Foundation. The Biotechnology Research Subcommittee will function in an advisory capacity to the Committee on Fundamental Science, the Director of the Office of Science and Technology Policy, and the Executive Office of the President. The Biotechnology Research Subcommittee will review the scientific aspects of proposed regulations and guidelines as they are developed.

The primary responsibilities of the Biotechnology Research Subcommittee are to: (i) Describe and review current Federal efforts in biotechnology research; (ii) identify and define the priority areas for future Federal biotechnology research, including areas needing greater emphasis, describing the role of each agency in those areas, and delineate where interagency cooperation would enhance progress in the biotechnology sciences, with an emphasis on integrated research efforts, where appropriate; (iii) assess major international efforts in the biotechnology sciences and develop mechanisms for international collaboration. For example, activities of the U.S.-European Community Task Force on Biotechnology have been coordinated through the Biotechnology Research Subcommittee; (iv) identify and review national and international policy issues (such as public education) associated with biotechnology; and (v) provide reviews, analyses, and recommendations to the Chairs of the Committee on Fundamental Science on scientific issues related to regulations and the applications of biotechnology research and biotechnology policies and issues.

In 1990, the Biotechnology Research Subcommittee replaced the Biotechnology Sciences Coordinating Committee. Both the Biotechnology Research Subcommittee and the Biotechnology Sciences Coordinating Committee previously functioned under the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET). While regulatory issues became the primary focus of the Biotechnology Sciences Coordinating Committee, the Biotechnology Research Subcommittee focuses on scientific issues, although it will still provide scientific support for regulatory responsibilities.

Appendix K. Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules

Appendix K specifies physical containment guidelines for large scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large scale research or production activities as specified in Section III-C–6. It is important to note that this appendix addresses only the biological hazard associated with organisms containing recombinant DNA. Other hazards accompanying the large scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this appendix.

All provisions shall apply to large scale research or production activities with the following modifications: (i) Appendix K shall supersede Appendix G when quantities in excess of 10 liters of culture are involved in research or production. Appendix K-II applies to Good Large Scale Practice; (ii) the institution shall appoint a Biological Safety Officer if it engages in large scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the Biological Safety Officer shall include those specified in Section IV-B–3; (iii) the institution shall establish and maintain a health surveillance program for personnel engaged in large scale research or production activities involving viable organisms containing recombinant DNA molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

Appendix K–I. Selection of Physical Containment Levels

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Section III. For purposes of large scale research or production, four physical containment levels are established. The four levels set containment conditions at those appropriate for the degree of hazard to health or the environment posed by the organism, judged by experience with similar organisms unmodified by recombinant DNA techniques and consistent with Good Large Scale Practice. The four biosafety levels of large scale physical containment are referred to as Good Large Scale Practice, BL1–Large Scale, BL2–Large Scale, and BL3–Large Scale. Good Large Scale Practice is recommended for large scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large scale use. Good Large Scale Practice is recommended for organisms such as those included in Appendix C which have built-in environmental limitations that permit optimum growth in the large scale setting but limited survival without adverse consequences in the environment. BL1–Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice. BL2–Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale. BL3–Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale. No provisions are made for large scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

Appendix K–II. Good Large Scale Practice (GLSP)

Appendix K–II–A. Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

Appendix K–II–B. Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the workplace is kept clean and orderly.

Appendix K–II–C. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g.,

uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area.

Appendix K–II–D. Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment.

Appendix K–II–E. Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations.

Appendix K–II–F. Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees.

Appendix K–II–G. The facility's emergency response plan shall include provisions for handling spills. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix K–III. Biosafety Level 1 (BL1)—Large Scale

Appendix K–III–A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K–III–B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G–II–A are met.

Appendix K–III–C. Culture fluids (except as allowed in Appendix K–III–D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–III–D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

Appendix K–III–E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–III–F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–III–G. Emergency plans required by Sections IV–B–2–b–(6) and IV–B–3–c–(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–IV. Biosafety Level 2 (BL2)—Large Scale

Appendix K–IV–A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K–IV–B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G–II–B are met.

Appendix K–IV–C. Culture fluids (except as allowed in Appendix K–IV–D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–IV–D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K–IV–E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–IV–F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the

organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–IV–G. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K–IV–H. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing sensing devices that monitor the integrity of containment during operations.

Appendix K–IV–I. A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K–IV–J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

Appendix K–IV–K. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K–IV–L. Emergency plans required by Sections IV–B–2–b–(6) and IV–B–3–c–(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–V. Biosafety Level 3 (BL3)—Large Scale

Appendix K–V–A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K–V–B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in Appendix G–II–C are met.

Appendix K–V–C. Culture fluids (except as allowed in Appendix K–V–D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–V–D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K–V–E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K–V–F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K–V–G. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

Appendix K–V–H. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K–V–I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

Appendix K–V–J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K–V–K. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities

involving viable organisms containing recombinant DNA molecules.

Appendix K–V–L. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K–V–M. Emergency plans required by Sections IV–B–2–b–(6) and IV–B–3–c–(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K–V–N. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

Appendix K–V–N–1. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

Appendix K–V–N–2. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

Appendix K–V–N–3. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

Appendix K–V–N–4. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

Appendix K–V–N–5. Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

Appendix K–V–N–6. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

Appendix K–V–N–7. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

Appendix K–V–N–8. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.

Appendix K–V–O. The following personnel and operational practices shall be required:

Appendix K–V–O–1. Personnel entry into the controlled area shall be through the entry area specified in Appendix K–V–N–1.

Appendix K–V–O–2. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

Appendix K–V–O–3. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

Appendix K-V-O–4. Persons under 18 years of age shall not be permitted to enter the controlled area.

Appendix K-V-O–5. The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

Appendix K-V-O–6. The controlled area shall be kept neat and clean.

Appendix K-V-O–7. Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

Appendix K-V-O–8. Animals and plants shall be excluded from the controlled area.

Appendix K-V-O–9. An effective insect and rodent control program shall be maintained.

Appendix K-V-O–10. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Serve doors leading directly outdoors shall be sealed and locked while work is in progress.

Appendix K-V–0–11. Persons shall wash their hands when exiting the controlled area.

Appendix K-V-O–12. Persons working in the controlled area shall be trained in emergency procedures.

Appendix K-V-O–13. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

Appendix K-V-O–14. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

Appendix K—Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL)—Large Scale (LS) Practice (see Appendix K–VI–A)

Criterion (See Appendix K–VI–B)	GLSP	BL1–LS	BL2–LS	BL3–LS
1. Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures.	K–II–A	G–I	G–I	G–I
2. Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees.	K–II–B	G–I ¶ 1	G–I ¶ 1	G–I ¶ 1
3. Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work.	K–II–C	G–II–A–1–h	G–II–B–2–f	G–II–C–2–i
4. Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place.	K–II–C	G–II–A–1–d G–II–A–1–e	G–II–B–1–d G–II–B–1–e	G–II–C–1–c G–II–C–1–d
5. Internal accident reporting	K–II–G	K–III–A	K–IV–A	K–IV–A

Criterion [See Appendix K–VI–B]	GLSP	BL1–LS	BL2–LS	BL3–LS
6. Medical surveillance	NR	NR	K–IV–A	K–V–A
7. Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment).	NR	K–III–B	K–IV–B	K–V–B
8. Culture fluids not removed from a system until organisms are inactivated.	NR	K–III–C	K–IV–C	K–V–C
9. Inactivation of waste solutions and materials with respect to their biohazard potential.	K–II–E	K–III–C	K–V–C	K–V–C
10. Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system.	Minimize Procedure K–II–F	Minimize Engineer K–III–B K–III–D	Prevent Engineer K–IV–B K–IV–D	Prevent Engineer K–V–B K–V–D
11. Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.	NR	Minimize K–III–E	Prevent K–IV–E	Prevent K–V–E
12. Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure.	NR	K–III–F	K–IV–F	K–V–F
13. Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features.	NR	NR	NR	K–V–G
14. Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.	NR	NR	Prevent K–IV–G	Prevent K–V–H
15. Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.	NR	NR	K–IV–H	K–V–I
16. Validated integrity testing of closed containment system	NR	NR	K–IV–I	K–V–J
17. Closed system to be permanently identified for record keeping purposes.	NR	NR	K–IV–J	K–V–K
18. Universal biosafety sign to be posted on each closed system	NR	NR	K–IV–K	K–V–L
19. Emergency plans required for handling large losses of cultures	K–II–G	K–III–G	K–IV–L	K–V–M
20. Access to the work place	NR	G–II–A–1–a	G–II–B–1–a	K–V–N
21. Requirements for controlled access area	NR	NR	NR	K–V–N&O

NR=not required.

Appendix K–VI. Footnotes of Appendix K

Appendix K–VI–A. This table is derived from the text in Appendices G and K and is not to be used in lieu of Appendices G and K.

Appendix K–VI–B. The criteria in this grid address only the biological hazards associated with organisms containing recombinant DNA. Other hazards accompanying the large scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

Appendix K–VII. Definitions to Accompany Containment Grid and Appendix K

Appendix K–VII–A. Accidental Release. An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

Appendix K–VII–B. Biological Barrier. A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

Appendix K–VII–C. Closed System. A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

Appendix K–VII–D. Containment. Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

Appendix K–VII–E. De minimis Release. *De minimis* release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

Appendix K–VII–F. Disinfection. Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

Appendix K–VII–G. Good Large Scale Practice Organism. For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, Recombinant DNA Safety Considerations, 1987, p. 34–35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large scale use or have built-in environmental limitations that permit optimum growth in the large scale setting but limited survival without adverse consequences in the environment; (ii) the recombinant DNA-engineered organism should be non-pathogenic, should be as safe in the large scale setting as the host organism, and without adverse consequences in the environment; and (iii) the vector/insert should be well characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms unknown to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

Appendix K–VII–H. Inactivation. Inactivation is any process that destroys

the ability of a specific microbiological agent or eukaryotic cell to self-replicate.

Appendix K–VII–I. Incidental Release. An incidental release is the discharge of a microbiological agent or eukaryotic cell from a containment system that is expected when the system is appropriately designed and properly operated and maintained.

Appendix K–VII–J. Minimization. Minimization is the design and operation of containment systems in order that any incidental release is a de minimis release.

Appendix K–VII–K. Pathogen. A pathogen is any microbiological agent or eukaryotic cell containing sufficient genetic information, which upon expression of such information, is capable of producing disease in healthy people, plants, or animals.

Appendix K–VII–L. Physical Barrier. A physical barrier is considered any equipment, facilities, or devices (e.g., fermentors, factories, filters, thermal oxidizers) which are designed to achieve containment.

Appendix K–VII–M. Release. Release is the discharge of a microbiological agent or eukaryotic cell from a containment system. Discharges can be incidental or accidental. Incidental releases are de minimis in nature; accidental releases may be de minimis in nature.

Appendix L. Release into the Environment of Certain Plants

Appendix L–I. General Information

Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Subcommittee, and specific approval by the NIH Director. Such experiments also require the approval of the Institutional Biosafety Committee before initiation.

Appendix L–II. Criteria Allowing Review by the RAC Plant Subcommittee Without the Requirement for Full RAC Review

In consultation with the RAC Plant Subcommittee and without the requirement for full RAC review (Institutional Biosafety Committee review and approval is necessary), NIH/ORDA may approve the growing of plants containing recombinant DNA in the field under the following conditions: (i) The plant species is a cultivated crop of a genus that has no species known to be a noxious weed; (ii) the introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants; (iii) the vector consists of DNA from exempt host-vector systems (see Appendix C), from plants of the same or closely related species, from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants, from plants pathogens only if sequences resulting in production of disease symptoms have been deleted, or chimeric vectors constructed from sequences of exempt host-vector systems (see Appendix C) or from sequences from plant pathogens in which the disease symptoms have been deleted. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests; (iv) plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the Institutional Biosafety Committee should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review and approval by the Institutional Biosafety Committee. Copies of such results must be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M. Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules Into the Genome of One or More Human Subjects

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH. Researchers not covered by the NIH Guidelines are encouraged to use Appendix M. Experiments in which recombinant DNA or DNA or RNA derived from recombinant DNA is introduced into one or more human subjects with the intent of stably modifying his/her genome are covered by Sections III–A–2, III–B–2, and III–B–3 (see Section V–U). Experiments in which recombinant DNA or DNA or RNA derived from recombinant DNA and that are not covered by Sections III–A–2, III–B–2, or III–B–3 and that are not considered exempt under Section V–U, are covered under Section III–C–7.

This document is intended to provide guidance in preparing proposals for NIH consideration under Sections III–A–2 and III–B–2. Section III–A–2 addresses Major Actions involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that have been determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. Proposals considered under Section III–A–2 will be reviewed by the RAC and approved by the NIH Director. RAC review of experiments considered under Section III–A–2 will follow publication of a precis of the proposal in the Federal Register and an opportunity for public comment. Section III–B–2 addresses Minor Actions involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that have been determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to qualify for the Accelerated Review process. Proposals considered under Sections III–A–2 and III–B–2 will be on a case-by-case basis. A list of actions approved under Sections III–A–2 and III–B–2 involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. The list of actions to the NIH Guidelines involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects does not include experiments considered to be exempt from RAC and NIH/ORDA review under Section III–C–7.

Since the recombinant DNA or DNA or RNA derived from recombinant DNA is expected to be confined following transfer to one or more human subjects,

no risk to public health or to the environment is expected. Nevertheless, Appendix M–I–B–4–b specifically asks the researchers to address this point.

This appendix will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed.

A proposal involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects will be considered by the RAC and/or NIH/ORDA only after the protocol has been approved by the local Institutional Biosafety Committee and Institutional Review Board in accordance with DHHS Regulations for the Federal Regulations for the Protection of Human Subjects (45 Code of Federal Regulations, Part 46). If a proposal involves children, special attention should be paid to subpart D of these DHHS regulations. The Institutional Review Board and Institutional Biosafety Committee may, at their discretion, condition their approval on further specific deliberation by the RAC and/or NIH/ORDA. Consideration of human gene transfer proposals by the RAC and/or NIH/ORDA may proceed simultaneously with review by other involved Federal agencies (see Appendix M–VII–A) provided that NIH/ORDA is notified of the simultaneous review. Meetings of the full RAC and its subcommittee will be open to the public except where trade secrets or proprietary information would be disclosed. The committee prefers that proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. Public review of these protocols will serve to inform the public about the technical aspects of the proposals as well as the meaning and significance of the research.

The clinical application of recombinant DNA techniques raises two general kinds of questions: (i) the questions usually discussed by Institutional Review Boards in their review of any proposed research involving one or more human subjects; and (ii) broader issues. The first type of question is addressed principally in Appendix M-I of this document. Several broader issues are discussed throughout Appendix M.

Appendix M-I requests a description of the protocol with special attention to the short-term risks and benefits of the proposed research to the patient and to other people, the selection of patients, informed consent, privacy, and confidentiality. Appendix M-II addresses special issues pertaining to the free flow of information about the clinical trials. These issues lie outside the usual purview of Institutional Review Boards and reflect general public concerns about biomedical research. Appendix M-III summarizes guidelines for submission of human gene transfer protocols for RAC review. Appendix M-IV specifies reporting requirements. Appendix M-V describes the procedures for Accelerated Review of human gene transfer experiments. Appendix M-VI describes the procedures to be followed for Expedited Review of single patient human gene transfer experiments. Appendix M-VII contains the footnotes to Appendix M.

The RAC will not at present entertain proposals for germ-line alterations but will consider for approval protocols involving somatic cell gene transfer. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning gene into a patient's somatic cells. In germ-line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring.

The acceptability of human somatic cell gene therapy has been addressed in several public documents as well as in numerous academic studies. In November 1982, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research published a report, Splicing Life, which resulted from a two-year process of public deliberations and hearing. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, Human Gene Therapy, which concluded: civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies. In light of this public support, the RAC is prepared to consider proposals for somatic cell gene therapy.

In its evaluation of proposals involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, the RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigations, namely, to protect the health and well-being of one or more human subjects being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) vertical transmission of genetic changes from an individual to his/her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and/or NIH/ORDA to assess the possibility that the proposed experiments will inadvertently affect reproductive cells or lead to infection of other people (e.g., medical personnel or relatives).

In recognition of the social concern that surrounds the subject of gene transfer, the RAC and NIH/ORDA will cooperate with other groups in assessing the possible long-term consequences of the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology.

* Responses to Appendix M should be provided in the form of either written answers or references to specific sections of the protocol or its appendices. Principal Investigators should indicate points which are not applicable with a brief explanation. Principal Investigators submitting proposals that employ essentially the same vector systems (or with minor variations), and/or that are based on the same preclinical testing as proposals previously reviewed by the RAC, may refer to preceding documents without having to rewrite such material.

Appendix M–I. Description of Proposal

Appendix M–I–A. Objectives and Rationale of the Proposed Research

State concisely the overall objectives and rationale of the proposed study. Provide information on the specific points that relate to whichever type of research is being proposed.

Appendix M–I–A–1. Use of Recombinant DNA for Therapeutic Purposes. For research in which recombinant DNA is transferred in order to treat a disease or disorder (e.g., genetic diseases, cancer, and metabolic

diseases), the following questions should be addressed:

Appendix M–I–A–1–a. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

Appendix M–I–A–1–b. Describe the natural history and range of expression of the disease selected for treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

Appendix M–I–A–1–c. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

Appendix M–I–A–1–d. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

Appendix M–I–A–2. Transfer of DNA for Other Purposes. Appendix M–I–A–2–a. Into what cells will the recombinant DNA be transferred?

Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?

Appendix M–I–A–2–b. What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?

Appendix M–I–B. Research Design, Anticipated Risks and Benefits

Appendix M–I–B–1. Structure and Characteristics of the Biological System. Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

Appendix M–I–B–1–a. What is the structure of the cloned DNA that will be used?

Appendix M–I–B–1–a–(1). Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

Appendix M–I–B–1–a–(2). What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)? From what source are these elements derived? Summarize what is currently known about the regulatory character of each element.

Appendix M–I–B–1–a–(3). Describe the steps used to derive the DNA construct.

Appendix M–I–B–1–b. What is the structure of the material that will be administered to the patient?

Appendix M–I–B–1–b–(1). Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M–I–B–1–b-(1)-(i) through (iii) are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?

Appendix M–I–B–1–b-(2). Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

Appendix M–I–B–2. Preclinical Studies, Including Risk-Assessment Studies. Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

Appendix M–I–B–2–a. Delivery System. Appendix M–I–B–2–a–(1). What cells are the intended target cells of recombinant DNA? What target cells are to be treated ex vivo and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

Appendix M–I–B–2–a–(2). Is the delivery system efficient? What percentage of the target cells contain the added DNA?

Appendix M–I–B–2–a–(3). How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

Appendix M–I–B–2–a–(4). How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

Appendix M–I–B–2–b. Gene Transfer and Expression. Appendix M–I–B–2–b–(1). What animal and cultured cell models were used in laboratory studies to assess the in vivo and in vitro efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

Appendix M–I–B–2–b–(2). What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

Appendix M–I–B–2–b–(3). Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system (see Appendix M–I–B–2–a) in achieving the minimally required level of gene transfer and expression (see Appendix M–I–B–2–b–(2)).

Appendix M–I–B–2–b–(4). To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

Appendix M–I–B–2–b–(5). In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

Appendix M–I–B–2–b–(6). Is the gene expressed in cells other than the target cells? If so, to what extent?

Appendix M–I–B–2–c. Retrovirus Delivery Systems. Appendix M–I–B–2–c–(1). What cell types have been infected with the retroviral vector

preparation? Which cells, if any, produce infectious particles?

Appendix M–I–B–2–c–(2). How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement of recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

Appendix M–I–B–2–c–(3). What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

Appendix M–I–B–2–c–(4). Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of the analyses?

Appendix M–I–B–2–c–(5). Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

Appendix M–I–B–2–d. Non-Retrovirus Delivery/Expression Systems. If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

Appendix M–I–B–3. Clinical Procedures, Including Patient Monitoring. Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

Appendix M–I–B–3–a. Will cells (e.g., bone marrow cells) be removed from patients and treated ex vivo? If so, describe the type, number, and intervals at which these cells will be removed.

Appendix M–I–B–3–b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

Appendix M–I–B–3–c. What treated cells (or vector/DNA combination) will be given to patients? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

Appendix M–I–B–3–d. How will it be determined that new gene sequences have been inserted into the patient's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

Appendix M–I–B–3–e. What studies will be conducted to assess the presence and effects of the contaminants?

Appendix M–I–B–3–f. What are the clinical endpoints of the study? Are there objections and quantitative measurements to assess the natural history of the disease? Will such measurements be used in patient follow-up? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will patient follow-up continue?

Appendix M–I–B–3–g. What are the major beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.

Appendix M–I–B–3–h. If a treated patient dies, what special post-mortem studies will be performed?

Appendix M–I–B–4. Public Health Considerations. Describe any potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:

Appendix M–I–B–4–a. On what basis are potential public health benefits or hazards postulated?

Appendix M–I–B–4–b. Is there a significant possibility that the added DNA will spread from the patient to other persons or to the environment?

Appendix M–I–B–4–c. What precautions will be taken against such spread (e.g., patients sharing a room, health-care workers, or family members)?

Appendix M–I–B–4–d. What measures will be undertaken to mitigate the risks, if any, to public health?

Appendix M–I–B–4–e. In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to patients? Are such concerns applicable to health care personnel?

Appendix M–I–B–5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities. Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:

Appendix M–I–B–5–a. What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format (see Appendix M–III–E).

Appendix M–I–B–5–b. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the patients. Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the patient?

Appendix M–I–C. Selection of the Patients

Estimate the number of patients to be involved in the proposed study. Describe recruitment procedures and patient eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable. Specifically:

Appendix M–I–C–1. How many patients do you plan to involve in the proposed study?

Appendix M–I–C–2. How many eligible patients do you anticipate being able to identify each year?

Appendix M–I–C–3. What recruitment procedures do you plan to use?

Appendix M–I–C–4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

Appendix M–I–C–5. How will patients be selected if it is not possible to include all who desire to participate?

Appendix M–I–D. Informed Consent

Indicate how patients will be informed about the proposed study and

how their consent will be solicited. The consent procedure should adhere to the requirements of DHHS regulations for the protection of human subjects (45 Code of Federal Regulations, Part 46). If the study involves pediatric or mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern include potential adverse effects, financial costs, privacy, long-term follow-up and post-mortem examination. When gene transfer is a procedure separate from a clinical protocol, Informed Consent documents shall be submitted for both the gene transfer and clinical protocols.

Appendix M–I–D–1. How will the major points covered in Appendices M–I–A through M–I–C be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them?

Appendix M–I–D–2. How will the innovative character and the theoretically possible adverse effects of the experiment be discussed with patients and/or parents or guardians? How will the potential adverse effects be compared with the consequences of the disease?

Appendix M–I–D–3. What explanation of the financial costs of the experiment, follow-up care, and any available alternatives will be provided to patients and/or parents or guardians?

Appendix M–I–D–4. How will patients and/or their parents or guardians be informed that the innovative character of the experiment may lead to great interest by the media in the research and in the treated patients?

Appendix M–I–D–5. How will the patients and/or their parents or guardians be informed about: (i) the irreversible consequences of some of the procedures performed? (ii) any adverse medical consequences that may occur if the subject(s) withdraws from the study once it has begun? (iii) expectations of willingness to cooperate in long-term follow-up? and (iv) expectations that permission to perform an autopsy will be granted in the event of a patient's death as a precondition for a patient's participation in the study? This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future patients.

Appendix M–I–E. Privacy and Confidentiality

Indicate what measure will be taken to protect the privacy of patients and their families as well as to maintain the confidentiality of research data.

Appendix M–I–E–1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed.

Appendix M–I–E–2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

Appendix M–II. Special Issues

Although the following issues are beyond the normal purview of local Institutional Review Boards, the RAC requests that Principal Investigators respond to Appendices M–II–A and M–II–B below:

Appendix M–II–A. What steps will be taken, consistent with Appendix M–I–E, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

Appendix M–II–B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among Principal Investigators and clinicians concerning research methods and results?

Appendix M–III. Guidelines for the Submission of Human Gene Transfer Protocols

Appendices M–III–A through M–III–D and M–IV apply to human gene transfer protocols considered under Section III–A–2 and III–B–2. Appendices M–III–A, M–IV, and M–V apply to human gene transfer protocols considered under Section III–B–2.

Appendix M–III–A. Principal Investigator-Submitted Material

Principal Investigators should submit the following materials to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M–III–A–1. Written proposals shall be submitted in the following order:

(1) Scientific abstract—1 page; (2) non-technical abstract—1 page; (3) Institutional Biosafety Committee and Institutional Review Board approvals and their deliberations pertaining to your protocol; (4) Response to Points to Consider—5 pages (see Appendix M through M–III); (6) protocol—20 pages excluding appendices—approved by the local Institutional Biosafety Committee and Institutional Review Board); (7) Informed Consent document—approved by the Institutional Review Board; (8) appendices including tables, figures, and manuscripts; (9) curricula vitae—2 pages for each key professional person in biographical sketch format; and (10) an indication of other Federal agencies to which the protocol is being submitted for review.

Appendix M–III–A–2. When a proposal has been submitted previously, there should be a short section (≤ 200 words) immediately following the abstracts that summarizes the major revisions since the last review.

Appendix M–III–A–3. Data provided shall include: (i) A description of the elements in the vector, (ii) the source of that information, (iii) the method by which sequence data were compiled, and (iv) three 3½ inch diskettes with the vector sequence in ASCII format.

Appendix M–III–B. Time Frame for Submissions

Note: Time frames are applicable only to protocols that are determined by NIH/ORDA to require full RAC review and NIH Director approval. Time frames do not apply to *Accelerated* Review human gene transfer experiments (see Section III–B–2 or those that only require registration with NIH/ORDA (see Section III–C–7).

Appendix M–III–B–1. Written material from Principal Investigator shall be submitted ≤ 8 weeks before the RAC meeting at which it will be reviewed.

Appendix M–III–B–2. Written comments from the primary reviewers to the Principal Investigator shall be submitted ≤ 4 weeks before the RAC meeting at which it will be reviewed.

Appendix M–III–B–3. Written responses (including critical data in response to the primary reviewers' comments) shall be submitted by the Principal Investigator to NIH/ORDA ≤ 2 weeks before the RAC meeting.

Appendix M–III–C. Oral Responses to the RAC

Principal Investigators shall limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted ≤ 2 weeks prior to the RAC meeting are prohibited.

Appendix M–III–D. Primary Reviewers' Responses

Appendix M–III–D–1. Primary Reviewers' Written Comments. The primary reviewers' written comments on a proposal should include the following:

Appendix M–III–D–1–a. Emphasize the issues related to gene marking, gene transfer, or gene therapy.

Appendix M–III–D–1–b. State explicitly whether the Points to Consider have been addressed satisfactorily.

Appendix M–III–D–1–c. Examine the scientific rationale, scientific context (relative to other proposals reviewed by the RAC), whether the preliminary in vitro and in vivo data were obtained in appropriate models and are sufficient, and whether questions related to safety, efficacy, and social/ethical context have been resolved.

Appendix M–III–D–1–d. Whenever possible, criticisms of Informed Consent documents should include written alternatives for suggested revisions for the RAC to consider.

Appendix M–III–D–1–e. Primary reviews should state whether the proposal is: (i) acceptable as written, (ii) expected to be acceptable with specific revisions or after satisfactory responses to specific questions raised on review, or (iii) unacceptable in its present form.

Appendix M–III–D–2. Oral Discussions by Primary Reviewers at the RAC Meeting. Appendix M–III–D–2–a. It should be possible for most primary reviewers to present their oral reviews in ≤ 5 minutes.

Appendix M–IV. Reporting Requirements

Appendix M–IV–A. Serious adverse effects of treatment should be reported immediately to the local Institutional Review Board, the NIH Office for Protection from Research Risks, and NIH/ORDA followed by the submission of a written report filed with each group. Reports submitted to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M–IV–B. Reports regarding the general progress of patients should be filed with both the local Institutional Review Board and NIH/ORDA within six months of the commencement of the experiment and at six-month intervals thereafter. These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects. In the event of a patient's death, a summary of the special post-mortem studies and statement of the cause of death should be submitted to the Institutional Review Board and NIH/ORDA, if available.

Appendix M–V. Procedures to the Followed for Accelerated Review of Human Gene Transfer Experiments by NIH/ORDA under Section III–B–2

Requests for Accelerated Review should be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M–V–A. Human gene transfer experiments in this category must be in accordance with the provisions of Section III–B–2. If the human gene transfer protocol does not qualify for Accelerated Review (see Section III–B–2) as determined by NIH/ORDA, then the Principal Investigator must submit the experiment for full RAC review and NIH approval in accordance with Section III–A–2.

Appendix M–V–B. No protocol shall be considered without Institutional Biosafety Committee and Institutional Review Board approval.

Appendix M–V–C. At this time, all gene transfer protocols must be considered experimental.

Appendix M–V–D. Principal Investigators requesting Accelerated Review (see Section III–B–2), must submit the relevant documentation in accordance with Appendix M–III. NIH/ORDA will notify the Principal Investigator whether the proposed study qualifies for the Accelerated Review process. If NIH/ORDA determines that an experiment does not qualify for Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review ≤ 8 weeks prior to the next scheduled RAC meeting.

Appendix M–V–E. It is expected that NIH/ORDA will consult with the RAC Chair and one or more RAC members, as necessary, when considering Accelerated Review human gene transfer protocols (see Section III–B–2).

Appendix M–V–F. The RAC Chair will provide a report on all human gene transfer protocols that have been approved by NIH/ORDA at the next regularly scheduled RAC meeting.

Appendix M–V–F–1. In accordance with Reporting Requirements (See Appendix M– IV), any adverse effects of the treatment should be reported immediately to the local Institutional Review Board, the NIH Office for Protection from Research Risks, and NIH/ORDA followed by the submission of a written report filed with each group. Reports submitted to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M–V–F–2. In accordance with Reporting Requirements (see Appendix M– IV), reports regarding the general progress of patients should be filed with both the local Institutional Review Board and NIH/ORDA within six months of the commencement of the experiment and at six-month intervals thereafter. In the event of a patient's death, a summary of the special post-mortem studies and statement of the cause of death should be submitted to the Institutional Review Board and NIH/ORDA, if available.

Appendix M–VI. Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments by NIH Director Under Section III–A–2 Requests for Expedited Review should be submitted to the Office of Recombinant DNA Activities, National Insitutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

Appendix M–VI–A. A Principal Investigator submitting a request to the NIH/ORDA for Expedited Review of a single patient gene transfer protocol shall provide detailed information regarding the necessity of Expedited Review.

Appendix M–VI–B. No protocol shall be considered without relevant Institutional Biosafety Committee and Institutional Review Board approvals.

Appendix M–VI–C. At this time, all gene transfer protocols are considered experimental.

Appendix M–VI–D. Regardless of the method of review, the Points to Consider is the standard of review for all gene transfer protocols.

Appendix M–VI–E. Review of such protocols may include intramural NIH experts but must include extramural experts.

Appendix M–VI–F. The reviewers shall consider similarity of the new protocol to previously approved protocols.

Appendix M–VI–G. The NIH/ORDA shall report to the RAC following Expedited Review and include all of the materials on which the decision was based. The RAC shall formally review the protocol at its next scheduled meeting. Patient privacy shall be maintained.

Appendix M–VI–H. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for Expedited Review. No protocol shall have more than one patient approved under Expedited Review.

Appendix M–VI–I. As requested in the context of non-expedited review, none of the costs of the experimental protocol shall be borne by the patient or the patient's family.

Appendix M–VI–J. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure.

Appendix M–VII. Footnotes of Appendix M

Appendix M–VII–A. The Food and Drug Administration has jurisdiction over products intended for use in human gene transfer clinical trials. For general information on the Food and Drug Administration's policies and regulatory requirements, see the Federal Register, Volume 51, pages 23309–23313, 1986.

Appendix M–VII–B. The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

Appendix P. Physical and Biological Containment for Recombinant DNA Research Involving Plants

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

Appendix P shall supersede Appendix G when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain Rhizobium species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) Are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P–I. General Plant Biosafety Levels

Appendix P–I–A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

Appendix P–I–B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix P–I–C. Four biosafety levels, referred to as Biosafety Level (BL) 1—Plants (P), BL2–P, BL3–P, and BL4–P, are established in Section II. The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in Section III. These biosafety levels are described in Appendix P–II. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P–I–D. BL1–P through BL4–P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in Appendix P–III, provide flexible approaches to ensure the safe conduct of research.

Appendix P–I–E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix P–III), if botanical reproductive structures are produced that have the potential of being released.

Appendix P–II. Physical Containment Levels

Appendix P–II–A. Biosafety Level 1—Plants (BL1–P)

Appendix P–II–A–1. Standard Practices (BL1–P)

Appendix P–II–A–1–a. Greenhouse Access (BL1–P)

Appendix P–II–A–1–a–(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P–II–A–1–a–(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1–P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P–II–A–1–b. Records (BL1–P)

Appendix P–II–A–1–b–(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–A–1–c. Decontamination and Inactivation (BL1–P)

Appendix P–II–A–1–c–(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P–II–A–1–d. Control of Undesired Species and Motile Macroorganisms (BL1–P)

Appendix P–II–A–1–d–(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P–II–A–1–d–(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P–II–A–1–e. Concurrent Experiments Conducted in the Greenhouse (BL1–P)

Appendix P–II–A–1–e–(1). Experiments involving other organisms that require a containment level lower than BL1–P may be conducted in the greenhouse concurrently with

experiments that require BL1–P containment, provided that all work is conducted in accordance with BL1–P greenhouse practices.

Appendix P–II–A–2. Facilities (BL1–P)

Appendix P–II–A–2–a. Definitions (BL1–P)

Appendix P–II–A–2–a–(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P–II–A–2–a–(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P–II–A–2–b. Greenhouse Design (BL1–P)

Appendix P–II–A–2–b–(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P–II–A–2–b–(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix P–II–B. Biosafety Level 2—Plants (BL2–P)

Appendix P–II–B–1. Standard Practices (BL2–P)

Appendix P–II–B–1–a. Greenhouse Access (BL2–P)

Appendix P–II–B–1–a–(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix P–II–B–1–a–(2). Personnel shall be required to read and follow instructions on BL2–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P–II–B–1–b. Records (BL2–P)

Appendix P–II–B–1–b–(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P–II–B–1–b–(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–B–1–b–(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P–II–B–1–c. Decontamination and Inactivation (BL2–P)

Appendix P–II–B–1–c–(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P–II–B–1–c–(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P–II–B–1–d. Control of Undesired Species and Motile Macroorganisms (BL2–P)

Appendix P–II–B–1–d–(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P–II–B–1–d–(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P–II–B–1–e. Concurrent Experiments Conducted in the Greenhouse (BL2–P)

Appendix P–II–B–1–e–(1). Experiments involving other organisms that require a containment level lower than BL2–P may be conducted in the greenhouse concurrently with experiments that require BL2–P containment provided that all work is conducted in accordance with BL2–P greenhouse practices.

Appendix P–II–B–1–f. Signs (BL2–P)

Appendix P–II–B–1–f–(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P–II–B–1–f–(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P–II–B–1–f–(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P–II–B–1–g. Transfer of Materials (BL2–P)

Appendix P–II–B–1–g–(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non–breakable container.

Appendix P–II–B–1–h. Greenhouse Practices Manual (BL2–P)

Appendix P–II–B–1–h–(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P–II–B–2. Facilities (BL2–P)

Appendix P–II–B–2–a. Definitions (BL2–P)

Appendix P–II–B–2–a–(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P–II–B–2–a–(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix P–II–B–2–b. Greenhouse Design (BL2–P)

Appendix P–II–B–2–b–(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P–II–B–2–b–(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P–II–B–2–c. Autoclaves (BL2–P)

Appendix P–II–B–2–c–(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P–II–B–2–d. Supply and Exhaust Air Ventilation Systems (BL2–P)

Appendix P–II–B–2–d–(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P–II–B–2–e. Other (BL2–P)

Appendix P–II–B–2–e–(1). BL2–P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Appendix P–II–C. Biosafety Level 3—Plants (BL3–P)

Appendix P–II–C–1. Standard Practices (BL3–P)

Appendix P–II–C–1–a. Greenhouse Access (BL3–P)

Appendix P–II–C–1–a–(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P–II–C–1–a–(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P–II–C–1–b. Records (BL3–P)

Appendix P–II–C–1–b–(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P–II–C–1–b–(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–C–1–b–(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P–II–C–1–c. Decontamination and Inactivation (BL3–P)

Appendix P–II–C–1–c–(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P–II–C–1–d. Control of Undesired Species and Motile Macroorganisms (BL3–P)

Appendix P–II–C–1–d–(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P–II–C–1–d–(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P–II–C–1–e. Concurrent Experiments Conducted in the Greenhouse (BL3–P)

Appendix P–II–C–1–e–(1). Experiments involving organisms that require a containment level lower than BL3–P may be conducted in the greenhouse concurrently with experiments that require BL3–P containment provided that all work is conducted in accordance with BL3–P greenhouse practices.

Appendix P–II–C–1–f. Signs (BL3–P)

Appendix P–II–C–1–f–(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P–II–C–1–f–(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

Appendix P–II–C–1–f–(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P–II–C–1–g. Transfer of Materials (BL3–P)

Appendix P–II–C–1–g–(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P–II–C–1–h. Greenhouse Practices Manual (BL3–P)

Appendix P–II–C–1–h–(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) Advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Appendix P–II–C–1–i. Protective Clothing (BL3–P)

Appendix P–II–C–1–i–(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P–II–C–1–i–(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P–II–C–1–j. Other (BL3–P)

Appendix P–II–C–1–j–(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P–II–C–1–j–(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P–II–C–2. Facilities (BL3–P)

Appendix P–II–C–2–a. Definitions (BL3–P)

Appendix P–II–C–2–a–(1). The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P–II–C–2–a–(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P–II–C–2–b. Greenhouse Design (BL3–P)

Appendix P–II–C–2–b–(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P–II–C–2–b–(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P–II–C–2–b–(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P–II–C–2–b–(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P–II–C–2–b–(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P–II–C–2–b–(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P–II–C–2–b–(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P–II–C–2–c. Autoclaves (BL3–P)

Appendix P–II–C–2–c–(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P–II–C–2–d. Supply and Exhaust Air Ventilation Systems (BL3–P)

Appendix P–II–C–2–d–(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P–II–C–2–d–(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80–85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52–68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P–II–C–2–e. Other (BL3–P)

Appendix P–II–C–2–e–(1). BL3–P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P–II–C–2–e–(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P–II–D. Biosafety Level 4—Plants (BL4–P)

Appendix P–II–D–1. Standard Practices (BL4–P)

Appendix P–II–D–1–a. Greenhouse Access (BL4–P)

Appendix P–II–D–1–a–(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P–II–D–1–a–(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P–II–D–1–a–(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P–II–D–1–a–(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P–II–D–1–a–(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4–P practices and procedures.

Appendix P–II–D–1–b. Records (BL4–P)

Appendix P–II–D–1–b–(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P–II–D–1–b–(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–D–1–b–(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P–II–D–1–b–(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P–II–D–1–c. Decontamination and Inactivation (BL4–P)

Appendix P–II–D–1–c–(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P–II–D–1–c–(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P–II–D–1–c–(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P–II–D–1–d. Control of Undesired Species and Motile Macroorganisms (BL4–P)

Appendix P–II–D–1–d–(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P–II–D–1–d–(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4–P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P–II–D–1–e. Concurrent Experiments Conducted in the Greenhouse (BL4–P)

Appendix P–II–D–1–e–(1). Experiments involving organisms that require a containment level lower than BL4–P may be conducted in the greenhouse concurrently with experiments that require BL4–P containment provided that all work is conducted in accordance with BL4–P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4–P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P–II–D–1–f. Signs (BL4–P)

Appendix P–II–D–1–f–(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P–II–D–1–f–(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

Appendix P–II–D–1–f–(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P–II–D–1–g. Transfer of Materials (BL4–P)

Appendix P–II–D–1–g–(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non- breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P–II–D–g–(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P–II–D–1–h. Greenhouse Practices Manual (BL4–P)

Appendix P–II–D–1–h–(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

Appendix P–II–D–1–i. Protective Clothing (BL4–P)

Appendix P–II–D–1–i–(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P–II–D–1–i–(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P–II–D–1–i–(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P–II–D–2. Facilities (BL4–P)

Appendix P–II–D–2–a. Greenhouse Design (BL4–P)

Appendix P–II–D–2–a–(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

Appendix P–II–D–2–a–(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P–II–D–2–a–(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P–II–D–2–a–(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P–II–D–2–a–(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P–II–D–2–a–(6). The walls, floors, and ceilings of the greenhouse

shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P–II–D–2–a–(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P–II–D–2–a–(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P–II–D–2–b. Autoclaves (BL4–P)

Appendix P–II–D–2–b–(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P–II–D–2–c. Supply and Exhaust Air Ventilation Systems (BL4–P)

Appendix P–II–D–2–c–(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P–II–D–2–c–(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P–II–D–2–d. Other (BL4–P)

Appendix P–II–D–2–d–(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P–II–D–2–d–(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P–II–D–2–d–(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P–II–D–2–d–(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P–III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P–III–A. Biological Containment Practices (Plants)

Appendix P–III–A–1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P–III–B. Biological Containment Practices (Microorganisms)

Appendix P–III–B–1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P–III–C. Biological Containment Practices (Macroorganisms)

Appendix P–III–C–1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) Use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

Appendix Q. Physical and Biological Containment for Recombinant DNA Research Involving Animals

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q–III–D). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

Appendix Q–I. General Considerations

Appendix Q–I–A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in Section III. For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2–N, BL3–N, and BL4–N and are described in the following sections of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

Appendix Q–I–B. Disposal of Animals (BL1–N through BL4–N)

Appendix Q–I–B–1. When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q–I–B–2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Appendix Q–II. Physical and Biological Containment Levels

Appendix Q–II–A. Biosafety Level 1—Animals (BL1–N)

Appendix Q–II–A–1. Standard Practices (BL1–N)

Appendix Q–II–A–1–a. Animal Facility Access (BL1–N)

Appendix Q–II–A–1–a–(1). The containment area shall be locked.

Appendix Q–II–A–1–a–(2). Access to the containment area shall be limited or restricted when experimental animals are being held.

Appendix Q–II–A–1–a–(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–A–1–b. Other (BL1–N)

Appendix Q–II–A–1–b–(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q–II–A–1–b–(2). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q–II–A–1–b–(3). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q–II–A–2. Animal Facilities (BL1–N)

Appendix Q–II–A–2–(a). Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix Q–II–B. Biosafety Level 2—Animals (BL2–N) (see Appendix Q–III–A)

Appendix Q–II–B–1. Standard Practices (BL2–N)

Appendix Q–II–B–1–a. Animal Facility Access (BL2–N)

Appendix Q–II–B–1–a–(1). The containment area shall be locked.

Appendix Q–II–B–1–a–(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–B–1–a–(3). The containment building shall be controlled and have a locking access.

Appendix Q–II–B–1–a–(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Appendix Q–II–B–1–a–(5). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q–II–B–1–b. Decontamination and Inactivation (BL2–N)

Appendix Q–II–B–1–b–(1). Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

Appendix Q–II–B–1–b–(2). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–B–1–c. Signs (BL2–N)

Appendix Q–II–B–1–c–(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q–II–B–1–d. Protective Clothing (BL2–N)

Appendix Q–II–B–1–d–(1). Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

Appendix Q–II–B–1–d–(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q–II–B–1–e. Records (BL2–N)

Appendix Q–II–B–1–e–(1). Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q–II–B–1–e–(2). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Appendix Q–II–B–1–f. Transfer of Materials (BL2–N)

Appendix Q–II–B–1–f–(1). Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q–II–B–1–g. Other (BL2–N)

Appendix Q–II–B–1–g–(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q–II–B–1–g–(2). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–B–1–g–(3). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Appendix Q–II–B–1–g–(4). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q–II–B–1–g–(5). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q–II–B–1–g–(6). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q–II–B–1–g–(7). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q–II–B–1–g–(8). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q–II–B–2. Animal Facilities (BL2–N)

Appendix Q–II–B–2–a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q–II–B–2–b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix Q–II–B–2–c. The animal containment area shall be designed so that it can be easily cleaned.

Appendix Q–II–B–2–d. Windows that open shall be fitted with fly screens.

Appendix Q–II–B–2–e. An autoclave shall be available for decontamination of laboratory wastes.

Appendix Q–II–B–2–f. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Appendix Q–II–C. Biosafety Level 3—Animals (BL3–N) (see Appendix Q–III–B)

Appendix Q–II–C–1. Standard Practices (BL3–N)

Appendix Q–II–C–1–a. Animal Facility Access (BL3–N)

Appendix Q–II–C–1–a–(1). The containment area shall be locked.

Appendix Q–II–C–1–a–(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–C–1–a–(3). The containment building shall be controlled and have a locking access.

Appendix Q–II–C–1–a–(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the

potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

Appendix Q–II–C–1–a–(5). Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Appendix Q–II–C–1–b. Decontamination and Inactivation (BL3–N)

Appendix Q–II–C–1–b–(1). The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q–II–C–1–b–(2). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q–II–C–1–b–(3). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–C–1–b–(4). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q–II–C–1–b–(5). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q–II–C–1–c. Signs (BL3–N)

Appendix Q–II–C–1–c–(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q–II–C–1–d. Protective Clothing (BL3–N)

Appendix Q–II–C–1–d–(1). Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3–N area and wearing of personal clothing.

Appendix Q–II–C–1–d–(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q–II–C–1–d–(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q–II–C–1–e. Records (BL3–N)

Appendix Q–II–C–1–e–(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q–II–C–1–e–(2). Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q–II–C–1–e–(3). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Appendix Q–II–C–1–f. Transfer of Materials (BL3–N)

Appendix Q–II–C–1–f–(1). Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q–II–C–1–f–(2). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q–II–C–1–g. Other (BL3–N)

Appendix Q–II–C–1–g–(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q–II–C–1–g–(2). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Appendix Q–II–C–1–g–(3). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q–II–C–1–g–(4). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q–II–C–1–g–(5). Experiments involving other organisms that require containment levels lower than BL3–N may be conducted in the same area concurrently with experiments requiring BL3–N containment, provided that they are conducted in accordance with BL3–N practices.

Appendix Q–II–C–1–g–(6). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q–II–C–1–g–(7). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q–II–C–1–g–(8). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q–II–C–1–g–(9). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q–II–C–1–g–(10). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q–II–C–1–g–(11). Personnel shall be required to shower before exiting the BL3–N area and wearing personal clothing.

Appendix Q–II–C–1–g–(12). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q–II–C–1–g–(13). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–C–1–g–(14). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q–II–C–2. Animal Facilities (BL3–N)

Appendix Q–II–C–2–a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q–II–C–2–b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q–II–C–2–c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Appendix Q–II–C–2–d. An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q–II–C–2–e. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Appendix Q–II–C–2–f. Access doors to the containment area shall be self-closing.

Appendix Q–II–C–2–g. The animal area shall be separated from all other areas.

Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

Appendix Q–II–C–2–h. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q–II–C–2–i. An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q–II–C–2–j. If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

Appendix Q–II–C–2–k. Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q–II–C–2–l. In lieu of open housing in the special animal room, animals held in a BL3–N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Appendix Q–II–C–2–m. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q–II–C–2–n. Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

Appendix Q–II–D. Biosafety Level 4—Animals (BL4–N) (see Appendix Q–III–C)

Appendix Q–II–D–1. Standard Practices (BL4–N)

Appendix Q–II–D–1–a. Animal Facility Access (BL4–N)

Appendix Q–II–D–1–a–(1). Individuals under 16 years of age shall not be permitted to enter the animal area.

Appendix Q–II–D–1–a–(2). The containment area shall be locked.

Appendix Q–II–D–1–a–(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–D–1–a–(4). The containment building shall be controlled and have a locking access.

Appendix Q–II–D–1–a–(5). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any

specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

Appendix Q–II–D–1–a–(6). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

Appendix Q–II–D–1–a–(7). Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

Appendix Q–II–D–1–a–(8). Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Appendix Q–II–D–1–b. Decontamination and Inactivation (BL4–N)

Appendix Q–II–D–1–b–(1). All contaminated liquid or solid wastes shall be decontaminated before disposal.

Appendix Q–II–D–1–b–(2). The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q–II–D–1–b–(3). All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

Appendix Q–II–D–1–b–(4). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q–II–D–1–b–(5). When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q–II–D–1–b–(6). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–D–1–b–(7). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

Appendix Q–II–D–1–b–(8). An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q–II–D–1–b–(9). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q–II–D–1–c. Signs (BL4–N)

Appendix Q–II–D–1–c–(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q–II–D–1–d. Protective Clothing (BL4–N)

Appendix Q–II–D–1–d–(1). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4–N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

Appendix Q–II–D–1–d–(2). A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q–II–D–1–d–(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q–II–D–1–e. Records (BL4–N)

Appendix Q–II–D–1–e–(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q–II–D–1–e–(2). A system shall be established for: (i) Reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q–II–D–1–e–(3). When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the

agents handled or the function of the facility.

Appendix Q–II–D–1–e–(4). A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Appendix Q–II–D–1–f. Transfer of Materials (BL4–N)

Appendix Q–II–D–1–f–(1). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q–II–D–1–f–(2). Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4–N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4–N animal facility to one with a lower containment classification.

Appendix Q–II–D–1–f–(3). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Appendix Q–II–D–1–g. Other (BL4–N)

Appendix Q–II–D–1–g–(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q–II–D–1–g–(2). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q–II–D–1–g–(3). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q–II–D–1–g–(4). Experiments involving other organisms that require containment levels lower than BL4–N may be conducted in the same area concurrently with experiments requiring BL4–N containment provided that they are conducted in accordance with BL4–N practices.

Appendix Q–II–D–1–g–(5). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q–II–D–1–g–(6). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q–II–D–1–g–(7). A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

Appendix Q–II–D–1–g–(8). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q–II–D–1–g–(9). The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

Appendix Q–II–D–1–g–(10). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q–II–D–1–g–(11). An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Appendix Q–II–D–1–g–(12). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q–II–D–1–g–(13). Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q–II–D–2. Animal Facilities (BL4–N)

Appendix Q–II–D–2–a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

Appendix Q–II–D–2–b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q–II–D–2–c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

Appendix Q–II–D–2–d. An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q–II–D–2–e. Access doors to the containment area shall be self-closing.

Appendix Q–II–D–2–f. All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

Appendix Q–II–D–2–g. The BL4–N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal

facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

Appendix Q–II–D–2–h. A necropsy room shall be provided within the BL4–N containment area.

Appendix Q–II–D–2–i. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q–II–D–2–j. A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q–II–D–2–k. Exhaust air from BL4–N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4–N containment area.

Appendix Q–II–D–2–l. All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1×10^{-3} micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80–120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

Appendix Q–II–D–2–m. If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1×10^{6} organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

Appendix Q–II–D–2–n. All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

Appendix Q–II–D–2–o. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q–II–D–2–p. Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

Appendix Q–II–D–2–q. The supply water distribution system shall be fitted with a back-flow preventer or break tank.

Appendix Q–II–D–2–r. All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

Appendix Q–II–D–2–s. Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

Appendix Q–III. Footnotes and References for Appendix Q

Appendix Q–III–A. If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

Appendix Q–III–B. Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3–N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

Appendix Q–III–C. Classes 4 and 5 microorganisms pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import Class 5 agents, special approval must be obtained from U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

Appendix Q–III–D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the

organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1–P through BL4–P containment practices and procedures as specified in Appendices G and P.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Effective Date: June 24, 1994.

Harold Varmus,

Director, National Institutes of Health.

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Friday
August 5, 1994

Part IV

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth three actions to be taken by the Director, National Institutes of Health (NIH), under the NIH Guidelines for Research Involving Recombinant DNA Molecules (Federal Register, July 5, 1994, Part IV).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: Today three actions are being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These three proposed actions were published for comment in the Federal Register of May 11, 1994 (59 FR 24618), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meeting on June 9–10, 1994.

I. Background Information and Decisions on Actions Under the NIH Guidelines

A. Amendment to Appendix M–I–D of the NIH Guidelines Regarding Informed Consent for Human Gene Transfer Protocols

During the December 2–3, 1993, Recombinant DNA Advisory Committee meeting, Dr. Gary Ellis, Director of the Office for Protection from Research Risks (OPRR), NIH, Bethesda, Maryland, responded to the written comments submitted by Dr. Zallen, Chair of the Working Group on Informed Consent Issues. Dr. Ellis noted the Recombinant DNA Advisory Committee's concern regarding specific issues that should be addressed in human gene transfer protocol Informed Consent documents, i.e., request for autopsy, recommendations for male/female contraception, separate Informed Consent documents when the gene therapy aspects of the protocols are independent of an ongoing clinical protocol, commitment to long-term patient follow-up, and financial responsibility of the institution for all research-related costs. During his presentation, Dr. Ellis provided the Recombinant DNA Advisory Committee with background information regarding the roles of both OPRR and local Institutional Review Boards (IRB) in the review of research proposals involving human subjects. Dr. Ellis recommended that the Recombinant DNA Advisory Committee draft a letter outlining its specific recommendations to OPRR for distribution and consideration by the local IRBs.

In a memorandum dated December 23, 1993, Dr. Ellis further clarified the avenues that should be pursued by the Recombinant DNA Advisory Committee with regard to translating its concern about the "quality and content of informed consent documents into constructive changes in the informed consent process," specifically in relation to human gene transfer. Dr. Ellis recommended that the NIH Guidelines should be amended to introduce consistency in Informed Consent document language.

During the March 3–4, 1994, Recombinant DNA Advisory Committee meeting, Dr. Doris Zallen, Chair of the Working Group on Informed Consent, provided a summary of the proposed amendments to Appendix M–I–D, Informed Consent. Two versions of revised Appendix M–I–D were presented: (1) the version drafted by the working group, and (2) a modified version incorporating the modifications suggested by Mr. Alex Capron. The Recombinant DNA Advisory Committee recommended that the working group should develop a consolidated version of Appendix M–I–D which includes language from both proposed documents. The Recombinant DNA Advisory Committee suggested that questions should be prefaced with an explanation as to the necessity for the requested information.

On April 27, 1994, Dr. Zallen submitted revised amendments to Appendix M–I– D, Informed Consent, in response to the specific comments posed by the Recombinant DNA Advisory Committee at its March 3–4, 1994, meeting. The proposed amendments were reviewed by the Recombinant DNA Advisory Committee during the June 9–10, 1994, meeting. The Recombinant DNA Advisory Committee approved a motion to accept the proposed amendments with minor editorial changes to Appendix M–I–D, Informed Consent, by a vote of 13 in favor, 0 opposed, and no abstentions.

The amended version of Appendix M–I–D, Informed Consent, reads:

Appendix M–I–D. Informed Consent

In accordance with the requirements of DHHS regulations for the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should also indicate how the Informed Consent document makes clear the special requirements of gene transfer research.

Appendix M–I–D–1. Communication About the Study to Potential Participants

Appendix M–I–D–1–a.

Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?

Appendix M–I–D–1–b.

How will the major points covered in Appendices M–I–A through M–I–C be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

Appendix M–I–D–1–c.

What is the length of time that potential participants will have to make a decision about their participation in the study?

Appendix M–I–D–1–d.

If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

Appendix M–I–D–2. Informed Consent Document

"Investigators submitting human gene transfer proposals for Recombinant DNA Advisory Committee review must include the Informed Consent document as approved by the local Institutional Review Boards. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a 'marker' or to enhance the power of immunotherapy for cancer.

"Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent process should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document.

Appendix M–I–D–2–a. General Requirements of Human Subjects Research

Appendix M–I–D–2–a–(1). Description/purpose of the study. "The subjects should be provided with a detailed explanation in non-

technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component.

Appendix M–I–D–2–a–(2). Alternatives. "The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches.

Appendix M–I–D–2–a–(3). Voluntary participation. "The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

Appendix M–I–D–2–a–(4). Benefits. "The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

Appendix M–I–D–2–a–(5). Possible risks, discomforts, and side effects. "There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization.

"If verbal descriptors (e.g., 'rare,' 'uncommon,' or 'frequent') are used to express quantitative information regarding risk, these terms should be explained.

"The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

"The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started.

Appendix M–I–D–2–a–(6). Costs. "The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved.

"Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury.

Appendix M–I–D–2–b. Specific Requirements of Gene Transfer Research

Appendix M–I–D–2–b–(1). Reproductive considerations. "To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

"The inclusion of pregnant or lactating women should be addressed.

Appendix M–I–D–2–b–(2). Long-term follow-up. "To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The principal investigator should request that subjects continue to provide a current address and telephone number.

"The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed.

Appendix M–I–D–2–b–(3). Request for autopsy. "To obtain vital information about the safety and efficacy of gene transfer, autopsies are to be performed, if feasible. Subjects should be informed that at the time of death, no matter what the cause, permission for any autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance.

Appendix M–I–D–2–b–(4). Interest of the media and others in the research. "To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (i) that the Institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (ii) that representatives of applicable Federal agencies (e.g., the National Institutes of Health and the Food and Drug Administration), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records."

I accept this recommendation, and Appendix M–I–D, Informed Consent, of the NIH Guidelines will be added accordingly.

B. Amendment to Appendix M–VI of the NIH Guidelines Regarding Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments

On April 29, 1994, Dr. Nelson Wivel of the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested that Appendix M–VI, Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments, be amended to clarify submission requirements.

The Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments currently reads:

"Appendix M–VI–D. Regardless of the method of review, the Points to Consider is the standard of review for all gene transfer protocols."

The proposed amendment reads:

"Appendix M–VI–D. Regardless of the method of review, the Points to Consider is the standard of review for all gene transfer protocols; therefore, submission of the response to the Points to Consider is required."

The proposed amendment was reviewed by the Recombinant DNA Advisory Committee during the June 9–10, 1994, meeting. The Recombinant DNA Advisory Committee approved a motion to accept the proposed amendment to Appendix M–VI, Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments, by a vote of 13 in favor, 0 opposed, and no abstentions.

I accept this recommendation, and Appendix M–VI, Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments, of the NIH Guidelines will be amended accordingly.

C. Deletion of Appendix L of the NIH Guidelines Regarding Release into the Environment

On April 29, 1994, Dr. Nelson Wivel of the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested that Appendix L, Release into the Environment of Certain Plants, be deleted from the NIH Guidelines based on the following: (1) Section I of the NIH Guidelines allows experiments to proceed that are reviewed and approved by another Federal agency that has jurisdiction for review and approval without the necessity for NIH review or approval (52 FR 31849); (2) the Recombinant DNA Advisory Committee has not reviewed any deliberate release experiment involving recombinant DNA since 1984; (3) at its May 30–31, 1991, meeting, the Recombinant DNA Advisory Committee recommended that Section III–A–2 be deleted from the NIH Guidelines; and (4) experiments involving deliberate release into the environment are currently reviewed within the framework of existing Federal regulations, as administered by the Environmental Protection Agency and the U.S. Department of Agriculture.

Section I of the NIH Guidelines was amended on August 24, 1987, such that any recombinant DNA experiment (other than human gene transfer) may

proceed without Recombinant DNA Advisory Committee and NIH approval if it has been reviewed and approved by another Federal agency that has jurisdiction over such a proposal. The amended version (52 FR 31849) of Section I reads as follows:

Section I–A. Purpose

"* * *Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, have been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval* * *."

On December 6, 1990, the Recombinant DNA Advisory Committee Planning Subcommittee recommended that the requirement for Recombinant DNA Advisory Committee review of experiments involving deliberate environmental release of organisms containing recombinant DNA be eliminated from the NIH Guidelines. This recommendation reflected the fact that the Federal regulatory agencies, the U.S. Department of Agriculture and the Environmental Protection Agency, are responsible for the review and approval of environmental release experiments. The Recombinant DNA Advisory Committee reviewed the request and recommended that the following sections be deleted from the NIH Guidelines (Actions Under the Guidelines, Federal Register, July 5, Part III):

Section III–A–2.

Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term "deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section III–A–2–a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendix L.

Section III–A–2–b. Deletion derivatives and single base changes not otherwise covered by the NIH Guidelines.

Section III–A–2–c. For extrachromosomal elements and microorganisms (including viruses), rearrangements and amplifications within a single genome. Rearrangements involving the introduction of DNA from different strains of the same species would not be covered by this exemption."

Based on these amendments to the NIH Guidelines, that have previously been recommended by the Recombinant DNA Advisory Committee, and the fact that the principles of planned introduction are now in place which provide a risk-assessment method by other Federal regulatory agencies, the Office of Recombinant DNA Activities requests that Appendix L be deleted from the NIH Guidelines.

Appendix L will be deleted as follows:

Appendix L. Release into the Environment of Certain Plants

Appendix L–I. General Information

Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Subcommittee, and specific approval by the NIH Director. Such experiments also require the approval of the Institutional Biosafety Committee before initiation.

Appendix L–II. Criteria Allowing Review by the RAC Plant Subcommittee Without the Requirement for Full RAC Review

"In consultation with the RAC Plant Subcommittee and without the requirement for full RAC review (Institutional Biosafety Committee review and approval is necessary), NIH/ORDA may approve the growing of plants containing recombinant DNA in the field under the following conditions: (i) the plant species is a cultivated crop of a genus that has no species known to be a noxious weed; (ii) the introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants; (iii) the vector consists of DNA from exempt host-vector systems (see Appendix C), from plants of the same or closely related species, from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants, from plant pathogens only if sequences resulting in production of disease symptoms have been deleted, or chimeric vectors constructed from sequences of exempt host-vector systems (see Appendix C) or from sequences from plant pathogens in which the disease symptoms have been deleted. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests; (iv) plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the Institutional Biosafety Committee should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review and approval by the Institutional Biosafety Committee. Copies of such results must be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838."

The proposed amendment was reviewed by the Recombinant DNA Advisory Committee during its June 9–10, 1994, meeting. The Recombinant DNA Advisory Committee approved a motion to accept the deletion of Appendix L, Regarding Release into the Environment, by a vote of 13 in favor, 0 opposed, and no abstentions.

I accept this recommendation, and Appendix L, Regarding Release into the Environment, of the NIH Guidelines will be deleted accordingly.

II. Summary of Actions

A. Amendment to Appendix M–I–D of the NIH Guidelines Regarding Informed Consent for Human Gene Transfer Protocols

Appendix M–I–D will read as follows:

Appendix M–I–D. Informed Consent

In accordance with the requirements of DHHS regulations for the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should also indicate how the Informed Consent document makes clear the special requirements of gene transfer research.

Appendix M–I–D–1. Communication About the Study to Potential Participants

Appendix M–I–D–1–a

Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?

Appendix M–I–D–1–b

How will the major points covered in Appendices M–I–A through M–I–C be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?

Appendix M–I–D–1–c

What is the length of time that potential participants will have to make a decision about their participation in the study?

Appendix M–I–D–1–d

If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?

Appendix M–I–D–2. Informed Consent Document

"Investigators submitting human gene transfer proposals for Recombinant DNA Advisory Committee review must include the Informed Consent document as approved by the local Institutional Review Boards. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a 'marker' or to enhance the power of immunotherapy for cancer.

"Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent document should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45 CFR 46). Indicate if each of the specified items appears in the Informed Consent document or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document.

Appendix M–I–D–2–a. General Requirements of Human Subjects Research

Appendix M–I–D–2–a–(1). Description/ Purpose of the Study

"The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component.

Appendix M–I–D–2–a–(2). Alternatives

"The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches.

Appendix M–I–D–2–a–(3). Voluntary Participation

"The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled.

Appendix M–I–D–2–a–(4). Benefits

"The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others.

Appendix M–I–D–2–a–(5). Possible Risks, Discomforts, and Side Effects

"There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization.

"If verbal descriptors (e.g., 'rare,' 'uncommon,' or 'frequent') are used to express quantitative information regarding risk, these terms should be explained.

"The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe.

"The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started.

Appendix M–I–D–2–a–(6). Costs

"The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved.

"Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury.

Appendix M–I–D–2–b. Specific Requirements of Gene Transfer Research

Appendix M–I–D–2–b–(1). Reproductive Considerations

"To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified.

"The inclusion of pregnant or lactating women should be addressed.

Appendix M–I–D–2–b–(2). Long-Term Follow-Up

"To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The principal investigator should request that subjects continue to provide a current address and telephone number.

"The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed.

Appendix M–I–D–2–b–(3). Request for Autopsy

"To obtain vital information about the safety and efficacy of gene transfer, subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance.

Appendix M–I–D–2–b–(4). Interest of the Media and Others in the Research

"To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (i) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (ii) that representatives of applicable Federal agencies (e.g., the National Institutes of Health and the Food and Drug Administration), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records."

B. Amendment to Appendix M–VI of the NIH Guidelines Regarding Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments

Appendix M–VI, Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments, will read as follows:

"Appendix M–VI–D. Regardless of the method of review, the Points to Consider is the standard of review for all gene transfer protocols; therefore, submission of the Points to Consider is required."

C. Deletion of Appendix L of the NIH Guidelines Regarding Release into the Environment

Appendix L, Release into the Environment of Certain Plants, be deleted in full: To see the full text of Appendix L, reference the background information and decisions on actions under the NIH Guidelines (Section I–C).

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists

in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

EFFECTIVE DATE: July 28, 1994.

Harold Varmus,

Director, National Institutes of Health.

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